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1) Griffith, EC, Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Dec 22 1998, 95 (26) p15183-8, ISSN 0027-8424 Journal Code: 7505876

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BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Design and Synthesis of Highly Potent Fumagillin Analogues from Homology Modeling for a Human MetAP-2

Cheol Kyu Han,[a,c] Soon Kil Ahn,[a,*] Nam Song Choi,[a] Ryung Kee Hong,[a] Seung Kee Moon,[a] Hyoung Sik Chun,[a] Sang Joon Lee,[a] Jung Woo Kim,[a] Chung Il Hong,[a] Deukjoon Kim,[b] Jeong Hyeok Yoon[c] and Kyoung Tai No[c,*]

[a]*Chong Kun Dang Research Institute 15-20, Osackdang-ri, Seonggeo-up, Cheon An, Chungcheongnamdo, 330-830, South Korea*
[b]*College of Pharmacy, Seoul National University, San 56-1, Shinrim-dong, Kwanak-gu, Seoul, 151-742, South Korea*
[c]*CAMD Research Center and Department of Chemistry, Soong Sil University, Seoul, 156-743, South Korea*

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Abstract—New fumagillin analogues were designed through structure-based molecular modeling with a human methionine aminopeptidase-2. Among the fumagillin analogues, cinnamic acid ester derivative **CKD-731** showed 1000-fold more potent proliferation inhibitory activity on endothelial cell than **TNP-470**.

Introduction

The concept of treating cancer by inhibition of angiogenesis (new blood vessel formation), which was proposed by Folkman,[1,2] is a promising strategy for cancer therapy.[3] Since Ingber et al. discovered that fumagillin (**1**) from *Aspergillus fumigatus* inhibits new blood vessel growth, many semisynthetic fumagillin analogues have been synthesized from fumagillol (**2**), the hydrolysis product of fumagillin.[4] Among these analogues, *O*-(chloroacetylcarbamoyl)fumagillol (TNP-470, AGM-1470) is currently in phase III clinical trials for the treatment of a variety of cancers.[5]

The underlying molecular mechanism of the inhibition of angiogenesis by these fumagillin derivatives remained unknown until Crews and Liu independently identified a fumagillin-binding protein, methionine aminopeptidase type 2 (MetAP-2).[6,7] Recently, Clardy and coworkers have reported the structure of a human MetAP-2 fumagillin complex, where the spiro-epoxide of fumagillin forms a covalent bond with the His231 in the active site of MetAP-2.[8] These reports have prompted us to disclose our study on the structure-based drug design of fumagillin analogues from homology modeling.[9]

In this communication, we wish to describe our effort to develop highly potent fumagillin analogues based on a 3D structure of human MetAP-2 by means of homology modeling.

Results and Discussion

Homology models

MetAPs are metal-dependent enzymes, and the crystal structure of *E. coli* MetAP-1 reveals that, two cobalt ions are ligated by two aspartic acids, a histidine and two glutamic acids in an active site. Five amino acids (**D, D, H, E** and **E**; boxed letters) which bind to two cobalts ions are conserved in both MetAP-1[10,11] and MetAP-2 (Fig. 1).[12–14] Based on these findings, we performed the homology modeling of human MetAP-2. The sequence alignments of *Pyrococcus furiosus* MetAP-2 and *E. coli* MetAP-1 showed a sequence identity of 35 and 28% to human MetAP-2, respectively. The five amino acids in the R_1–R_5 domains are also conserved in *P. furiosus* MetAP-2 (Fig. 1). A three dimensional model of human MetAP-2 was built from known X-ray coordinates of *E. coli* MetAP-1 (PDB: 1MAT) and *P. furiosus* MetAP-2 (PDB: 1XGS) using MODELER.[15]

Keywords: Antitumor compounds; molecular modeling/mechanics.
*Corresponding author. E-mail: skahn@ckd.co.kr

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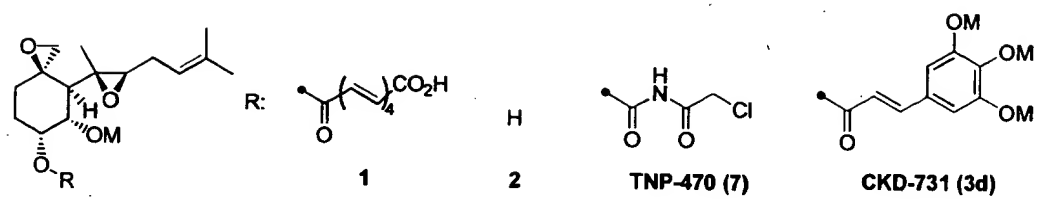


Structure optimization of fumagillin derivatives using structure-based design

With the human MetAP-2 model, a series of fumagillin derivatives **1–7** were designed (Table 1). In designing the inhibitors, the distance between the methylene carbon of the spiro-epoxide moiety of the fumagillin and the NH group of His231 was constrained to 3 Å because they form a covalent bond in the complex. The docked structure of human MetAP-2 and **CKD-731**, the most potent inhibitor of the series is illustrated in Figure 2. In the complex, a water molecule is bound to two cobalt ions. The distances between the cobalt ions and the water oxygen are 1.95 Å and 1.97 Å. The same water forms a hydrogen bond with the oxygen of the spiro-epoxide of **CKD-731**, which might facilitate the opening of the epoxide ring. The structure also reveals another water molecule which forms hydrogen bonds with the oxygens of the methoxy group and the epoxide of the alkyl side chain of **CKD-731**. This water also plays a role in stabilizing His231. A pocket formed with His339, Ile338, Phe219 and Tyr444 is almost fully occupied by the terminal isopropylidene group. Since there are a well-defined hydrophobic valley formed with Leu328 and Leu447, and a large pocket which is connected to this valley and surrounded by Asn327, Val374, Asp376 and His375, we designed fumagillin analogues with functional groups which can bind efficiently to this valley and pocket. To optimize hydrophobic interactions with the valley and van der Waals contacts at the pocket, we introduced several functional groups as described in Table 1. We have designed fumagillin derivatives by introducing functional groups with an aromatic ring which can interact hydrophobically with the Leu447 of human MetAP-2. Cinnamic acid esters **3b–3e**, phenylalkanoic acid esters **3f–3g**, benzyl carbamates **4a–4d** and benzyl carbonates **5a–5c** have been designed. For comparison purposes, alkyl acid ester **3a** and xanthate **6** were also prepared.

Synthesis of fumagillin derivatives

Fumagillol (**2**) was acylated with NaH and an acid chloride to provide compound **3**.[5] Treatment of the known phenoxycabonyl fumagillol with amine produced the carbamate **4**. Preparation of carbonate **5** was achieved by coupling of fumagillol (**2**) with benzyl chloroformate. Xanthate derivative **6** was prepared from **2** using CS_2 and benzyl bromide (Scheme 1).

Biological assays

Antiproliferating activities of fumagillin derivatives were evaluated against calf pulmonary artery (SPAE, ATTIC HRL 209) endothelial cells, lymphoma EL-4 cells and murine leukemia P388D1. IC_{50} values were colorimetrically measured by SRB (CPAE cell) or MTT (EL-4, P388D) methods. The biological data for compounds **1–7** are shown in Table 1.

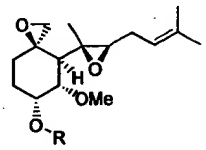
Structure–activity relationships

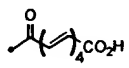
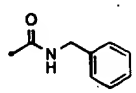
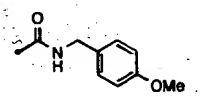
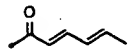
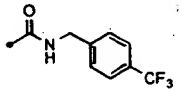
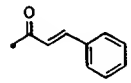
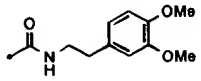
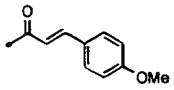
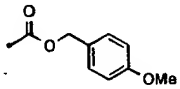
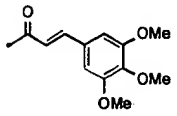
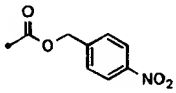
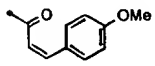
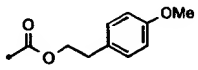
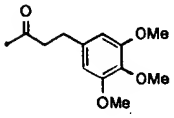
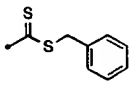
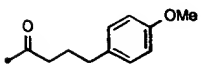
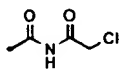
Among the designed compounds, the cinnamic acid esters **3b–3d** and benzyl carbamates **4a–4d** showed more potent activity than TNP-470, while the phenylalkanoic acid esters **3f–3g** and benzyl carbonates **5a–5c** were less active. It seems that the aromatic ring should be positioned to contact with Leu447 for maximizing hydrophobic interaction. The *trans*-cinnamic acid esters have an optimum fixed geometry for the hydrophobic interaction with Leu447. The activity tends to decrease as the bonds between the carbonyl group and the aromatic ring in the group R are more freely rotatable. In the docking model of the phenylalkanoic acid esters **3f–3g** and benzyl carbonate derivatives **5a–5c**, the aromatic rings point away from the Leu447. To support this assumption, we also prepared *cis*-cinnamic acid ester derivative **3e** which has an aromatic ring but cannot interact with Leu447.[16] The cell proliferation inhibitory

		R_1		R_2			R_3		R_4			R_5			
E.-coli MetAP-1	I VN I	D	VTV I KDGFHG	D	TSKM	EYCG	H	GIGR	FTI	E	PMV	AQY	E	HT I V	295
Human MetAP-1	I VNV	D	I TLYRNGYHG	D	LNDF	SYCG	H	GIHK	FTI	E	PMI	AQF	E	HTL L	394
Rat MetAP-2	I CK I	D	FGTH I SGR I I	D	CAFT	NLNG	H	SIGP	YAI	E	TFG	AQF	E	HT I L	480
Human MetAP-2	I CK I	D	FGTH I SGR I I	D	CAFT	NLNG	H	SIGQ	YAI	E	TFG	AQF	E	HT I L	478
P.f. MetAP-2	YLK I	D	VGVH I DGFTA	D	TAVT	NLSG	H	KIER	FAI	E	PEA	AQF	E	HT I V	295

Figure 1. Sequence alignment of the R_1–R_5 domains of MetAP-1 and MetAP-2.

Table 1. In vitro cell proliferation inhibitory activity of fumagillin derivatives against lymphoma EL-4 cell, calf pulmonary artery endothelial (CPAE) cell and murine leukemia P388D1[a]



	Subst. R	EL-4	CPAE	P388D1		Subst. R	EL-4	CPAE	P388D1
1		0.42	0.37	>1	4a		0.01	0.07	>1
2	H	5.16	15	>10	4b		0.042	0.044	>1
3a		5.05	12	>1	4c		0.014	0.0054	>1
3b		0.05	0.046	>1	4d		0.042	0.044	>1
3c		0.00019	0.00006	>1	5a		42	18	ND
3d		0.00015	0.00003	>1	5b		2.47	0.11	>1
3e		12	24	ND[b]	5c		153	2.3	ND
3f		36	44	ND	6		14	42	ND
3g		83	125	ND	7		0.03	0.04	>10

[a]IC_{50} values in ng/mL.
[b]ND: not determined.

activity of the *cis*-cinnamic acid ester derivative **3e** was 10^5 fold less than that of the *trans*-cinnamic acid ester derivatives **3b–3d**. Moreover the xanthate **6** was less active than the carbonyl ester derivatives **3b–3d**.

In summary, a human MetAP-2 model was built through homology modeling and several potent inhibitors were designed based on this model structure. Although the cell-level assay results do not always

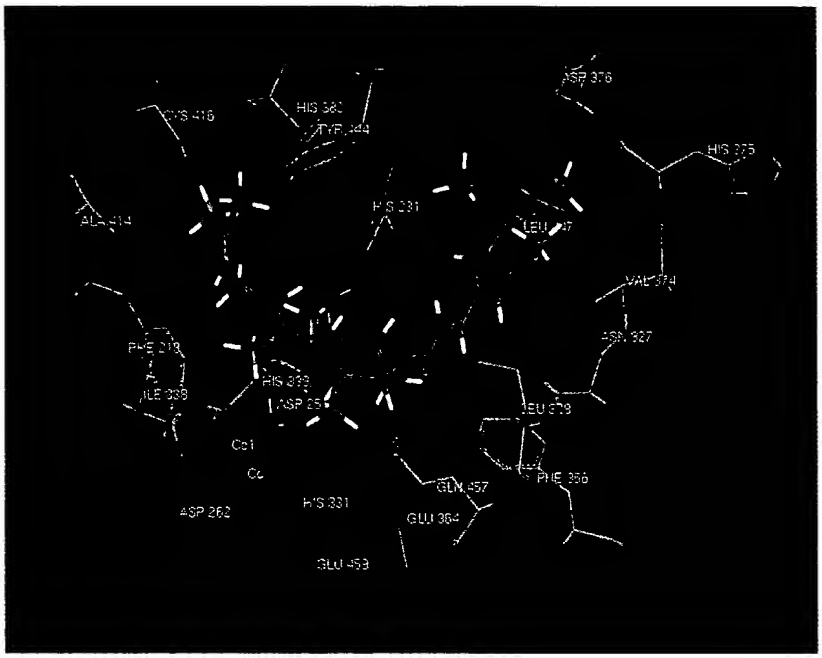
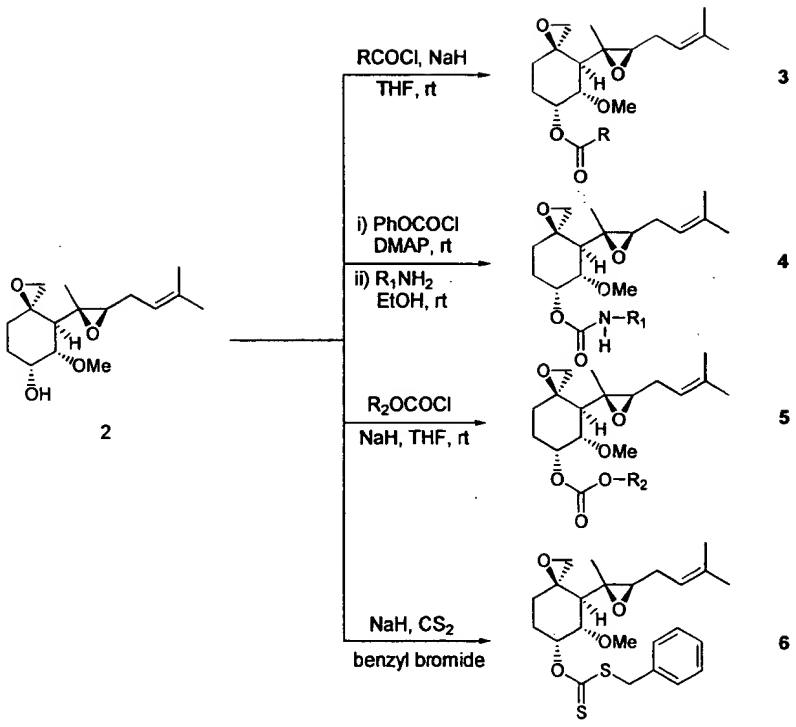


Figure 2. The docked structure of **CKD-731** complex with MetAP-2.



Scheme 1.

reflect the molecular-level interactions between enzyme and ligands, this study does provide some clues to designing fumagillin analogues as antiangiogenic anticancer agents.

Acknowledgements

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Molecular recognition of angiogenesis inhibitors fumagillin and ovalicin by methionine aminopeptidase 2.

Griffith E C; Su Z; Niwayama S; Ramsay C A; Chang Y H; Liu J O

Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.

Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Dec 22 **1998**, 95 (26) p15183-8, ISSN 0027-8424 Journal Code: 7505876

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Angiogenesis inhibitors are a novel class of promising therapeutic agents for treating cancer and other human diseases. Fumagillin and ovalicin compose a class of structurally related natural products that potently inhibit angiogenesis by blocking endothelial cell proliferation. A synthetic analog of fumagillin, TNP-470, is currently undergoing clinical trials for treatment of a variety of cancers. A common target for fumagillin and ovalicin recently was identified as the type 2 methionine aminopeptidase (**MetAP2**). These natural products bind **MetAP2**

covalently, inhibiting its enzymatic activity. The specificity of this binding is underscored by the lack of inhibition of the closely related type 1 enzyme, **MetAP1**. The molecular basis of the high affinity and specificity of these inhibitors for **MetAP2** has remained undiscovered. To determine the structural elements of these inhibitors and **MetAP2** that are involved in this interaction, we synthesized fumagillin analogs in which each of the potentially reactive epoxide groups was removed either individually or in combination. We found that the ring epoxide in fumagillin is involved in the covalent modification of **MetAP2**, whereas the side chain epoxide group is dispensable. By using a fumagillin analog tagged with fluorescein, His-231 in **MetAP2** was identified as the residue that is covalently modified by fumagillin. Site-directed mutagenesis of His-231 demonstrated its importance for the catalytic activity of **MetAP2** and confirmed that the same residue is covalently modified by fumagillin. These results, in agreement with a recent structural study, suggest that fumagillin and ovalicin inhibit **MetAP2** by irreversible blockage of the active site.

Griffith E C; Su Z; Niwayama S; Ramsay C A; Chang Y H; Liu J O
Dec 22 **1998**,

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       10201902  2
            111  METHIONINE(W)AMINOPEPTIDASE(2N)2
      S5    128  METAP(2N)2 OR METAP2 OR METHIONINE(W)AMINOPEPTIDASE(2N)2
? s variant or analogue?? or substitut? or modifi? or muta?
Processing
         148657  VARIANT
         179771  ANALOGUE??
         646500  SUBSTITUT?
         915253  MODIFI?
        1127067  MUTA?
      S6 2766758  VARIANT OR ANALOGUE?? OR SUBSTITUT? OR MODIFI? OR MUTA?
? s s5 and s6
            128  S5
        2766758  S6
      S7     47  S5 AND S6
? s s7 and py<=2001
Processing
Processing
             47  S7
       40175417  PY<=2001
      S8     39  S7 AND PY<=2001
? rd
>>>Duplicate detection is not supported for File 340.

>>>Records from unsupported files will be retained in the RD set.
...completed examining records
      S9     21  RD (unique items)
? t s9/3,k,ab/1-21

 9/3,K,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12940365   21567734   PMID: 11711298
    QSAR of the inhibition of angiogenesis by TNP-470 and ovalicin
analogues: another example of an allosteric interaction.
  Mekapati S B; Hansch C
  Department of Chemistry, Pomona College, Claremont, CA 91711, USA.
  Bioorganic & medicinal chemistry (England)  Dec 2001,  9  (12)
 p3225-30,  ISSN 0968-0896   Journal Code: 9413298
  Contract/Grant No.: ROIES07595; ES; NIEHS
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: Completed
  QSAR have been formulated for variations of TNP-470 and Ovalicin on
various cell lines. In the examples of mouse lymphocyte cells and bovine
endothelial cells the results suggest an allosteric interaction. These
results are compared with the binding of nitrobenzene to hemoglobin in rats
in vivo. Such a reaction does not occur with methionine aminopeptidase.
```

QSAR of the inhibition of angiogenesis by TNP-470 and ovalicin **analogues**: another example of an allosteric interaction.
Dec **2001**,
Enzyme No.: EC 3.4.- (**methionine aminopeptidase 2**);
EC 3.4.11 (Aminopeptidases); EC 3.4.24 (Metalloendopeptidases)
Chemical Name: Angiogenesis Inhibitors; Enzyme Inhibitors; Sesquiterpenes ; O-(chloroacetylcarbamoyl)fumagillol; ovalicin; **methionine aminopeptidase 2**; Aminopeptidases; Metalloendopeptidases

9/3,K,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11348687 21415419 PMID: 11524009
Steady-state kinetic characterization of substrates and metal-ion specificities of the full-length and N-terminally truncated recombinant human methionine aminopeptidases (type 2).
Yang G; Kirkpatrick R B; Ho T; Zhang G F; Liang P H; Johanson K O; Casper D J; Doyle M L; Marino J P; Thompson S K; Chen W; Tew D G; Meek T D
Department of Assay Methodology Development, GlaxoSmithKline Pharmaceuticals, 709 Swedeland Road, King of Prussia, Pennsylvania 19406, USA. guang yang@sbphrd.com
Biochemistry (United States) Sep 4 **2001**, 40 (35) p10645-54,
ISSN 0006-2960 Journal Code: 0370623
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The steady-state kinetics of a full-length and truncated form of the type **2** human **methionine aminopeptidase** (hMetAP2) were analyzed by continuous monitoring of the amide bond cleavage of various peptide substrates and methionyl **analogues** of 7-amido-4-methylcoumarin (AMC) and p-nitroaniline (pNA), utilizing new fluorescence-based and absorbance-based assay substrates and a novel coupled-enzyme assay method. The most efficient substrates for hMetAP2 appeared to be peptides of three or more amino acids for which the values of k(cat)/K(m) were approximately 5 x 10(5) M(-1) min(-1). It was found that while the nature of the P1' residue of peptide substrates dictates the substrate specificity in the active site of hMetAP2, the P2' residue appears to play a key role in the kinetics of peptidolysis. The catalytic efficiency of dipeptide substrates was found to be at least 250-fold lower than those of the tripeptides. This substantially diminished catalytic efficiency of hMetAP2 observed with the alternative substrates MetAMC and MetpNA is almost entirely due to the reduction in the turnover rate (k(cat)), suggesting that cleavage of the amide bond is at least partially rate-limiting. The 107 N-terminal residues of hMetAP2 were not required for either the peptidolytic activity of the enzyme or its stability. Steady-state kinetic comparison and thermodynamic analyses of an N-terminally truncated form and full-length enzyme yielded essentially identical kinetic behavior and physical properties. Addition of exogenous Co(II) cation was found to significantly activate the full-length hMetAP2, while Zn(II) cation, on the other hand, was unable to activate hMetAP2 under any concentration that was tested.

Sep 4 **2001**,
The steady-state kinetics of a full-length and truncated form of the type **2** human **methionine aminopeptidase** (hMetAP2) were analyzed by continuous monitoring of the amide bond cleavage of various peptide substrates and methionyl **analogues** of 7-amido-4-methylcoumarin (AMC) and p-nitroaniline (pNA), utilizing new fluorescence-based and...
; Amino Acid **Substitution**; Aminopeptidases --antagonists and inhibitors--AI; Aminopeptidases--chemistry--CH; Aminopeptidases--genetics --GE; Anilides; Cations, Divalent; Circular...
Enzyme No.: EC 3.4.- (**methionine aminopeptidase 2**);

EC 3.4.11 (Aminopeptidases); EC 3.4.24 (Metalloendopeptidases)
Chemical Name: Anilides; Cations, Divalent; Isoenzymes; Metals; Recombinant Proteins; norleucine p-nitroanilide; Norleucine; **methionine aminopeptidase 2**; Aminopeptidases; Metalloendopeptidases

9/3,K,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10971902 20516078 PMID: 11060750
TNP-470: an angiogenesis inhibitor in clinical development for cancer.
Kruger E A; Figg W D
National Cancer Institute/NIH, Medicine Branch, 9000 Rockville Pike, Bethesda, MD 20892, USA.
Expert opinion on investigational drugs (ENGLAND) Jun **2000**, 9 (6) p1383-96, ISSN 1354-3784 Journal Code: 9434197
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
TNP-470, an **analogue** of fumagillin, has been shown to inhibit angiogenesis in vitro and in vivo. In 1992, TNP-470 entered clinical development for cancer as an anti-angiogenic agent. It is currently in Phase I/II trials in Kaposi's sarcoma, renal cell carcinoma, brain cancer, breast cancer, cervical cancer and prostate cancer. In early clinical reports, TNP-470 is tolerated up to 177 mg/m(2) with neurotoxic effects (fatigue, vertigo, ataxia, and loss of concentration) being the principal dose limiting toxicity (DLT). Terminal half-life values are short and have shown intermittent and intrapatient variation (range: 0.05 - 1.07 h). Recently, mechanistic studies have identified cell cycle mediators and the protein **methionine aminopeptidase-2** (**MetAP-2**) as molecular targets of TNP-470 and fumagillin. Animal studies confirm some toxic effects on normal angiogenic processes such as the female reproductive system and wound healing, which will require caution and close monitoring in the clinic. TNP-470 is one of the first anti-angiogenic compounds to enter clinical trials, making it a valuable prototype for future trials of angiogenesis inhibitors in oncology.

Jun **2000**,
TNP-470, an **analogue** of fumagillin, has been shown to inhibit angiogenesis in vitro and in vivo. In 1992...

... 05 - 1.07 h). Recently, mechanistic studies have identified cell cycle mediators and the protein **methionine aminopeptidase-2** (**MetAP-2**) as molecular targets of TNP-470 and fumagillin. Animal studies confirm some toxic effects on...

9/3,K,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10967925 20530186 PMID: 11079802
cis-fumagillin, a new **methionine aminopeptidase** (type **2**) inhibitor produced by Penicillium sp. F2757.
Kwon J Y; Jeong H W; Kim H K; Kang K H; Chang Y H; Bae K S; Choi J D; Lee U C; Son K H; Kwon B M
Korea Research Institute of Bioscience and Biotechnology, Yusong, Taejon, Republic of Korea.
Journal of antibiotics (JAPAN) Aug **2000**, 53 (8) p799-806, ISSN 0021-8820 Journal Code: 0151115
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM

Record type: Completed
Selective inhibition against the yeast **MetAP2** (**methionine aminopeptidase** type **2**) was detected in the fermentation broth of a fungus F2757 that was later identified as Penicillium janczewskii. A new compound cis-fumagillin methyl ester (1) was isolated from the diazomethane treated fermentation extracts together with the known compound fumagillin methyl ester (2). The cis-fumagillin methyl ester, a stereoisomer of fumagillin methyl ester at the C2'-C3' position of the aliphatic side chain, selectively inhibited growth of the map1 **mutant** yeast strain (MetAP1 deletion strain) at a concentration as low as 1 ng. However, the wild type yeast w303 and the **mutant** map2 (**MetAP2** deleted) strains were resistant up to 10 microg of the compound. In enzyme experiments, compound 1 inhibited the **MetAP2** with an IC50 value of 6.3 nM, but it did not inhibit the MetAP1 (IC50 >200 microM). Compound 2 also inhibited the **MetAP2** with an IC50 value of 9.2 nM and 105 microM against MetAP1.

cis-fumagillin, a new **methionine aminopeptidase** (type **2**) inhibitor produced by Penicillium sp. F2757.
Aug **2000**,
Selective inhibition against the yeast **MetAP2** (**methionine aminopeptidase** type **2**) was detected in the fermentation broth of a fungus F2757 that was later identified as...

... the C2'-C3' position of the aliphatic side chain, selectively inhibited growth of the map1 **mutant** yeast strain (MetAP1 deletion strain) at a concentration as low as 1 ng. However, the wild type yeast w303 and the **mutant** map2 (**MetAP2** deleted) strains were resistant up to 10 microg of the compound. In enzyme experiments, compound 1 inhibited the **MetAP2** with an IC50 value of 6.3 nM, but it did not inhibit the MetAP1 (IC50 >200 microM). Compound 2 also inhibited the **MetAP2** with an IC50 value of 9.2 nM and 105 microM against MetAP1.
Enzyme No.: EC 3.4.- (**methionine aminopeptidase 2**); EC 3.4.11 (Aminopeptidases); EC 3.4.24 (Metalloendopeptidases)
Chemical Name: Epoxy Compounds; Fatty Acids, Unsaturated; fumagillin methyl ester; **methionine aminopeptidase 2**; Aminopeptidases; Metalloendopeptidases

9/3,K,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10730074 20263284 PMID: 10805534
Two continuous spectrophotometric assays for methionine aminopeptidase.
Zhou Y; Guo X C; Yi T; Yoshimoto T; Pei D
Department of Chemistry, The Ohio State University, Columbus 43210, USA.
Analytical biochemistry (UNITED STATES) Apr 10 **2000**, 280 (1) p159-65, ISSN 0003-2697 Journal Code: 0370535
Contract/Grant No.: AI40575; AI; NIAID
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Two spectrophotometric assays have been developed for methionine aminopeptidases (MetAPs). The first method employs a thioester substrate which, upon enzymatic removal of the N-terminal methionine, generates a free thiol group. The released thiol is quantitated using Ellman's reagent. The MetAP reaction is conveniently monitored on a UV-VIS spectrophotometer in a continuous fashion, with the addition of an excess of Ellman's reagent into the assay reaction. Two tripeptide **analogues** were synthesized and found to be excellent substrates of both Escherichia coli MetAP and human **MetAP2** (k(cat)/K(M) = 2.8 x 10(5) M(-1) s(-1) for the most reactive substrate). In the second assay method, the MetAP reaction is coupled to a prolyl aminopeptidase reaction using Met-Pro-p-nitroanilide as

substrate. MetAP-catalyzed cleavage of the N-terminal methionine produces prolyl-p-nitroanilide, which is rapidly hydrolyzed by the prolyl aminopeptidase from Bacillus coagulans to release a chromogenic product, p-nitroaniline. This allows the MetAP reaction to be continuously monitored at 405 nm on a UV-VIS spectrophotometer. The assays have been applied to determine the pH optima and kinetic constants for the E. coli and human MetAPs as well as to screen MetAP inhibitors. These results demonstrate that the current assays are convenient, rapid, and sensitive methods for kinetic studies of MetAPs and effective tools for screening MetAP inhibitors.

Apr 10 **2000**,
...the addition of an excess of Ellman's reagent into the assay reaction. Two tripeptide **analogues** were synthesized and found to be excellent substrates of both Escherichia coli MetAP and human **MetAP2** (k(cat)/K(M) = 2.8 x 10(5) M(-1) s(-1) for the...

9/3,K,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10702508 20225886 PMID: 10760954
Selective inhibition of endothelial cell proliferation by fumagillin is not due to differential expression of methionine aminopeptidases.
Wang J; Lou P; Henkin J
Cancer Research, Pharmaceutical Product Division, Abbott Laboratories Abbott Park, Illinois 60064, USA. jieyi.wang@abbott.com
Journal of cellular biochemistry (UNITED STATES) Apr **2000**, 77 (3) p465-73, ISSN 0730-2312 Journal Code: 8205768
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The angiogenesis inhibitors fumagillin and TNP-470 selectively inhibit the proliferation of endothelial cells, as compared with most other cell types. The mechanism of this selective inhibition remains uncertain, although **methionine aminopeptidase-2** (**MetAP2**) has recently been found to be a target for fumagillin or TNP-470, which inactivates **MetAP2** enzyme activity through covalent **modification** . Primary cultures of human endothelial cells and six other non-endothelial cell types were treated with fumagillin to determine its effect on cell proliferation. Only the growth of endothelial cells was completely inhibited at low concentrations of fumagillin. MetAP1 and **MetAP2** levels in these cells were investigated to determine whether differential enzyme expression plays a role in the selective action of fumagillin. Western blot analysis and RT-PCR data showed that MetAP1 and **MetAP2** were both expressed in these different types of cells, thus, ruling out differential expression of MetAP1 and **MetAP2** as an explanation for the cell specificity of fumagillin. Expression of **MetAP2** , but not of MetAP1, is regulated. Treatment of human microvascular endothelial cells (HMVEC) with fumagillin resulted in threefold increases of **MetAP2** protein in the cells, while MetAP1 remained constant. Similar upregulation of **MetAP2** by exposure to fumagillin was also observed in non-endothelial cells, eliminating this response as an explanation for cell specificity. Taken together, these results indicate that while **MetAP2** plays a critical role in the effect of fumagillin on endothelial cell proliferation, differential endogenous expression or fumagillin-induced upregulation of methionine aminopeptidases is not responsible for this observed selective inhibition.

Apr **2000**,
... compared with most other cell types. The mechanism of this selective inhibition remains uncertain, although **methionine aminopeptidase%%**

%-2 (MetAP2) has recently been found to be a target for fumagillin or TNP-470, which inactivates MetAP2 enzyme activity through covalent modification. Primary cultures of human endothelial cells and six other non-endothelial cell types were treated...

... the growth of endothelial cells was completely inhibited at low concentrations of fumagillin. MetAP1 and MetAP2 levels in these cells were investigated to determine whether differential enzyme expression plays a role...

... selective action of fumagillin. Western blot analysis and RT-PCR data showed that MetAP1 and MetAP2 were both expressed in these different types of cells, thus, ruling out differential expression of MetAP1 and MetAP2 as an explanation for the cell specificity of fumagillin. Expression of MetAP2, but not of MetAP1, is regulated. Treatment of human microvascular endothelial cells (HMVEC) with fumagillin resulted in threefold increases of MetAP2 protein in the cells, while MetAP1 remained constant. Similar upregulation of MetAP2 by exposure to fumagillin was also observed in non-endothelial cells, eliminating this response as an explanation for cell specificity. Taken together, these results indicate that while MetAP2 plays a critical role in the effect of fumagillin on endothelial cell proliferation, differential endogenous...

Enzyme No.: EC 3.4.- (methionine aminopeptidase 2); EC 3.4.11 (Aminopeptidases); EC 3.4.11.18 (methionyl aminopeptidase); EC 3.4...

Chemical Name: Antibiotics; Fatty Acids, Unsaturated; fumagillin; methionine aminopeptidase 2; Aminopeptidases; methionyl aminopeptidase; Metalloendopeptidases

9/3,K,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

1/28

10581604 20100194 PMID: 10636239

Design and synthesis of highly potent fumagillin analogues from homology modeling for a human MetAP-2.

Han C K; Ahn S K; Choi N S; Hong R K; Moon S K; Chun H S; Lee S J; Kim J W; Hong C I; Kim D; Yoon J H; No K T

Chong Kun Dang Research Institute, Chungcheongnamdo, South Korea.

Bioorganic & medicinal chemistry letters (ENGLAND) Jan 3 2000, 10 (1) p39-43, ISSN 0960-894X Journal Code: 9107377

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

New fumagillin analogues were designed through structure-based molecular modeling with a human methionine aminopeptidase-2. Among the fumagillin analogues , cinnamic acid ester derivative CKD-731 showed 1000-fold more potent proliferation inhibitory activity on endothelial cell than TNP-470.

Design and synthesis of highly potent fumagillin analogues from homology modeling for a human MetAP-2.

Jan 3 2000,

New fumagillin analogues were designed through structure-based molecular modeling with a human methionine aminopeptidase-2. Among the fumagillin analogues , cinnamic acid ester derivative CKD-731 showed 1000-fold more potent proliferation inhibitory activity on...

Enzyme No.: EC 3.4.- (methionine aminopeptidase 2); EC 3.4.11 (Aminopeptidases); EC 3.4.24 (Metalloendopeptidases)

Chemical Name: Angiogenesis Inhibitors; CKD 731; Cinnamates; Epoxy Compounds; Fatty Acids, Unsaturated; Growth Inhibitors; fumagillin;

methionine aminopeptidase 2; **Aminopeptidases; Metalloendopeptidases**

9/3,K,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10295658 99284515 PMID: 10354468
A methionine aminopeptidase and putative regulator of translation initiation is required for cell growth and patterning in Drosophila.
Cutforth T; Gaul U
Laboratory of Developmental Neurogenetics, The Rockefeller University, 1230 York Avenue, Box 248, New York, NY 10021, USA.
Mechanisms of development (IRELAND) Apr 1999, 82 (1-2) p23-8, ISSN 0925-4773 Journal Code: 9101218
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have isolated mutations **in the gene Drosophila** methionine aminopeptidase 2 **(DMAP2), which encodes a homolog of the type** 2 methionine aminopeptidase **from yeast, also known as the eukaryotic initiation factor 2alpha (eIF2alpha) associated protein p67. Weak DMAP2** mutations **cause ommatidial rotation defects and loss of ventral tissue in the compound eye as well as extra wing veins, whereas stronger alleles impair tissue growth. These limited phenotypes, in conjunction with the differential accumulation of DMAP2 transcripts throughout embryonic and larval development, suggest that a subset of proteins is spatially and temporally regulated at the level of post-translational processing or translation initiation during development. These results provide genetic evidence for post-transcriptional control in the development of multicellular organisms.**

Apr 1999,
We have isolated mutations **in the gene Drosophila** methionine aminopeptidase 2 **(DMAP2), which encodes a homolog of the type** 2 methionine aminopeptidase **from yeast, also known as the eukaryotic initiation factor 2alpha (eIF2alpha) associated protein p67. Weak DMAP2** mutations **cause ommatidial rotation defects and loss of ventral tissue in the compound eye as well...**
...; development--GD; Eye--growth and development--GD; Genes, Insect; Glycoproteins--genetics--GE; Molecular Sequence Data; Mutation; **Peptide Chain Initiation; Phenotype; Sequence Homology, Amino Acid; Wing --growth and development--GD**

9/3,K,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10102044 99079987 PMID: 9860943
Molecular recognition of angiogenesis inhibitors fumagillin and ovalicin by methionine aminopeptidase 2.
Griffith E C; Su Z; Niwayama S; Ramsay C A; Chang Y H; Liu J O
Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Dec 22 1998, 95 (26) p15183-8, ISSN 0027-8424 Journal Code: 7505876
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Angiogenesis inhibitors are a novel class of promising therapeutic agents for treating cancer and other human diseases. Fumagillin and ovalicin

compose a class of structurally related natural products that potently inhibit angiogenesis by blocking endothelial cell proliferation. A synthetic analog of fumagillin, TNP-470, is currently undergoing clinical trials for treatment of a variety of cancers. A common target for fumagillin and ovalicin recently was identified as the type 2 methionine aminopeptidase (MetAP2). These natural products bind MetAP2 covalently, inhibiting its enzymatic activity. The specificity of this binding is underscored by the lack of inhibition of the closely related type 1 enzyme, MetAP1. The molecular basis of the high affinity and specificity of these inhibitors for MetAP2 has remained undiscovered. To determine the structural elements of these inhibitors and MetAP2 that are involved in this interaction, we synthesized fumagillin analogs in which each of the potentially reactive epoxide groups was removed either individually or in combination. We found that the ring epoxide in fumagillin is involved in the covalent modification of MetAP2, whereas the side chain epoxide group is dispensable. By using a fumagillin analog tagged with fluorescein, His-231 in MetAP2 was identified as the residue that is covalently modified by fumagillin. Site-directed mutagenesis of His-231 demonstrated its importance for the catalytic activity of MetAP2 and confirmed that the same residue is covalently modified by fumagillin. These results, in agreement with a recent structural study, suggest that fumagillin and ovalicin inhibit MetAP2 by irreversible blockage of the active site.

Molecular recognition of angiogenesis inhibitors fumagillin and ovalicin by methionine aminopeptidase 2.
Dec 22 1998,
... of cancers. A common target for fumagillin and ovalicin recently was identified as the type 2 methionine aminopeptidase (MetAP2). These natural products bind MetAP2 covalently, inhibiting its enzymatic activity. The specificity of this binding is underscored by the lack...

...enzyme, MetAP1. The molecular basis of the high affinity and specificity of these inhibitors for MetAP2 has remained undiscovered. To determine the structural elements of these inhibitors and MetAP2 that are involved in this interaction, we synthesized fumagillin analogs in which each of the...

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...; DE; Fatty Acids, Unsaturated--chemistry--CH; Histidine; Metalloendopeptidases--antagonists and inhibitors--AI; Metalloendopeptidases--chemistry--CH; Mutagenesis, Site-Directed; Recombinant Proteins --antagonists and inhibitors--AI; Recombinant Proteins--chemistry--CH; Recombinant Proteins--metabolism...
Enzyme No.: EC 3.4.- (methionine aminopeptidase 2); EC 3.4.11 (Aminopeptidases); EC 3.4.24 (Metalloendopeptidases)
Chemical Name: Antibiotics; Fatty Acids, Unsaturated; Recombinant Proteins; Sesquiterpenes; ovalicin; fumagillin; Cysteine; Histidine; methionine aminopeptidase 2; Aminopeptidases; Metalloendopeptidases

9/3,K,AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10023673 99001036 PMID: 9784858

Synthetic analogues of TNP-470 and ovalicin reveal a common molecular basis for inhibition of angiogenesis and immunosuppression.

Turk B E; Su Z; Liu J O

Center for Cancer Research, Massachusetts Institute of Technology, Cambridge 02139, USA.

Bioorganic & medicinal chemistry (ENGLAND) Aug 1998, 6 (8) p1163-9, ISSN 0968-0896 Journal Code: 9413298

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

TNP-470 (1), a synthetic derivative of the natural product fumagillin (2), potently inhibits angiogenesis in vivo and the growth of endothelial cell cultures in vitro. The structurally related natural product ovalicin (3) also inhibits angiogenesis but possesses potent immunosuppressive activity. The recent finding that all three drugs bind and inhibit the same target, methionine aminopeptidase 2 (MetAP2), raised the question of whether TNP-470 is also immunosuppressive and whether inhibition of MetAP2 underlies both activities of ovalicin. To address these questions, we synthesized a series of analogues of TNP-470 and ovalicin and tested them for their abilities to inhibit the proliferation of either endothelial cell or mixed lymphocyte cultures. TNP-470 and its analogues were found to possess both immunosuppressive and anti-angiogenic activities. A strong correlation was observed between the ability of compounds to inhibit bovine and human endothelial cell growth and their ability to inhibit the mouse mixed lymphocyte reaction (MLR), implying that the two activities share a common molecular basis, i.e., inhibition of MetAP2. Interestingly, ovalicin and several other compounds behaved differently in the human MLR than in either the mouse MLR or human endothelial cell proliferation assays, pointing to possible species-specific and cell type-specific differences in the metabolism or uptake of these compounds.

Synthetic analogues of TNP-470 and ovalicin reveal a common molecular basis for inhibition of angiogenesis and...

Aug 1998,

... immunosuppressive activity. The recent finding that all three drugs bind and inhibit the same target, methionine aminopeptidase 2 (MetAP2), raised the question of whether TNP-470 is also immunosuppressive and whether inhibition of MetAP2 underlies both activities of ovalicin. To address these questions, we synthesized a series of analogues of TNP-470 and ovalicin and tested them for their abilities to inhibit the proliferation of either endothelial cell or mixed lymphocyte cultures. TNP-470 and its analogues were found to possess both immunosuppressive and anti-angiogenic activities. A strong correlation was observed...

... MLR), implying that the two activities share a common molecular basis, i.e., inhibition of MetAP2. Interestingly, ovalicin and several other compounds behaved differently in the human MLR than in either...

9/3,K,AB/11 (Item 11 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

09623786 98035818 PMID: 9367524

A dominant negative mutation in Saccharomyces cerevisiae methionine aminopeptidase-1 affects catalysis and interferes with the function of methionine aminopeptidase-2.

Klinkenberg M; Ling C; Chang Y H

School of Medicine, Saint Louis University Health Sciences Center, 1402 South Grand Boulevard, St. Louis, Missouri 63104, USA.

Archives of biochemistry and biophysics (UNITED STATES) Nov 15 1997, 347 (2) p193-200, ISSN 0003-9861 Journal Code: 0372430
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Methionine aminopeptidase (MetAP) enzymes require the metal ion cobalt, but little is known about the role of cobalt in the structural stability or catalysis of these enzymes. In Escherichia coli MetAP, for which a crystal structure is available, the five amino acid residues liganding the two cobalt ions are Asp97, Asp108, His171, Glu204, and Glu235. These five amino acids are conserved in all MetAPs sequenced to date. The C-terminal domain of the yeast Saccharomyces cerevisiae MetAP1 is 41% identical to E. coli MetAP and contains these cobalt coordinating residues. Using site-directed mutagenesis on the gene coding for yeast MetAP1, we replaced Asp219 (corresponding to Asp97 in E. coli MetAP) with Asn. The yeast D219N mutant enzyme has 10(3)-fold lower catalytic activity and a different substrate specificity when compared to wild-type yeast MetAP1. These results indicate that the side-chain of Asp219 is important for catalysis. Expression of D219N-MetAP1 in yeast causes a slow-growth phenotype and interferes with wild-type MetAP1 in a dominant manner. Expression of D219N-MetAP1 also affects the function of S. cerevisiae MetAP2.

A dominant negative mutation in Saccharomyces cerevisiae methionine aminopeptidase-1 affects catalysis and interferes with the function of methionine aminopeptidase-2.
Nov 15 1997,

... 41% identical to E. coli MetAP and contains these cobalt coordinating residues. Using site-directed mutagenesis on the gene coding for yeast MetAP1, we replaced Asp219 (corresponding to Asp97 in E. coli MetAP) with Asn. The yeast D219N mutant enzyme has 10(3)-fold lower catalytic activity and a different substrate specificity when compared...

... in a dominant manner. Expression of D219N-MetAP1 also affects the function of S. cerevisiae MetAP2.
Descriptors: Aminopeptidases--genetics--GE; *Aminopeptidases--metabolism --ME; *Cobalt--metabolism--ME; *Metalloendopeptidases--metabolism--ME; * Mutation; *Saccharomyces cerevisiae--genetics--GE...; genetics--GE; Binding Sites; Escherichia coli--enzymology--EN; Escherichia coli --genetics--GE; Genes, Fungal; Kinetics; Mutagenesis, Site-Directed; Recombinant Proteins--metabolism--ME; Saccharomyces cerevisiae--enzymology --EN; Substrate Specificity
Enzyme No.: EC 3.4.- (methionine aminopeptidase 2); EC 3.4.11 (Aminopeptidases); EC 3.4.11.18 (methionyl aminopeptidase); EC 3.4...
Chemical Name: Recombinant Proteins; Aspartic Acid; Cobalt; methionine aminopeptidase 2; Aminopeptidases; methionyl aminopeptidase; Metalloendopeptidases

9/3,K,AB/12 (Item 1 from file: 55)
DIALOG(R)File 55:Biosis Previews(R)

13342336 BIOSIS NO.: 200100549485
Substituted beta-amino acid inhibitors of methionine aminopeptidase-2.
AUTHOR: Craig Richard A(a); Henkin Jack; Kawai Megumi; Lynch Linda M; Patel Jyoti; Sheppard George S; Wang Jieyi
AUTHOR ADDRESS: (a)Racine, WI**USA
JOURNAL: Official Gazette of the United Stat s Patent and Trademark Office Patents 1247 (1):pNo Pagination June 5, 2001
MEDIUM: e-file

ISSN: 0098-1133
DOCUMENT TYPE: Patent
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A class of substituted b-amino acids are potent inhibitor of methionine aminopeptidase type 2 (MetAP2) and are thus useful in inhibiting angiogenesis and disease conditions which depend upon angiogenesis for their development such as diabetic retinopathy, tumor growth, and conditions of inflammation. Pharmaceutical compounds containing the compounds and methods of inhibiting methionine aminopeptidase-2, and angiogenesis are also disclosed.

2001

Substituted beta-amino acid inhibitors of methionine aminopeptidase-2.
2001

ABSTRACT: A class of substituted b-amino acids are potent inhibitor of methionine aminopeptidase type 2 (MetAP2) and are thus useful in inhibiting angiogenesis and disease conditions which depend upon angiogenesis for...

...tumor growth, and conditions of inflammation. Pharmaceutical compounds containing the compounds and methods of inhibiting methionine aminopeptidase-2, and angiogenesis are also disclosed.
DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: methionine aminopeptidase-2
--...

...substituted beta-amino acid inhibitors

9/3,K,AB/13 (Item 2 from file: 55)
DIALOG(R)File 55:Biosis Previews(R)

13284968 BIOSIS NO.: 200100492117
Redirecting the specific reactivity of a natural product and its application to functional proteomics.
AUTHOR: Tamiya Junko(a); Cravatt Benjamin F; Sorensen Erik J(a)
AUTHOR ADDRESS: (a)Department of Chemistry, Skaggs Institute for Chemical Biology, Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA, 92037: jtamiya@scripps.edu**USA
JOURNAL: Abstracts of Papers American Chemical Society 222 (1-2):pBIOL90
2001
MEDIUM: print
CONFERENCE/MEETING: 222nd National Meeting of the American Chemical Society Chicago, Illinois, USA August 26-30, 2001
SPONSOR: American Chemical Society
ISSN: 0065-7727
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Activity-based protein profiling aims to create chemical agents to profile changes in enzyme activity in complex proteomes. Combining this methodology with a natural product scaffold, a library of biotinylated analogs of the natural product fumagillin was constructed and tested against complex proteomes. Fumagillin is an angiogenesis inhibitor, which contains an electrophilic spiroexpoxide and a hydrophobic side chain. The spiroepoxide covalently modifies the

metalloprotease methionine aminopeptidase-2 (MetAp-2). Variation of the side chain to both hydrophobic and hydrophilic moieties redirected this natural product, facilitating the specific labeling of a diverse number of proteins directly in complex proteomes.

2001

2001

...ABSTRACT: angiogenesis inhibitor, which contains an electrophilic spiroexpoxide and a hydrophobic side chain. The spiroepoxide covalently modifies the metalloprotease methionine aminopeptidase-2 (MetAp-2). Variation of the side chain to both hydrophobic and hydrophilic moieties redirected this natural product...
DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: ...metalloprotease methionine aminopeptidase-2;

9/3,K,AB/14 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

10270603 Genuine Article#: 506ZD Number of References: 42
Title: Protection of translation initiation factor eIF2 phosphorylation correlates with eIF2-associated glycoprotein p67 levels and requires the lysine-rich domain I of p67 (ABSTRACT AVAILABLE)
Author(s): Datta R; Choudhury P; Bhattacharya M; Leon FS; Zhou Y; Datta B (REPRINT)
Corporate Source: Kent State Univ,Dept Chem,Kent//OH/44242 (REPRINT); Kent State Univ,Dept Chem,Kent//OH/44242; Univ Nebraska,Ctr Biotechnol,Lincoln//NE/68588
Journal: BIOCHIMIE, 2001, V83, N10 (OCT), P919-931
ISSN: 0300-9084 Publication date: 20011000
Publisher: EDITIONS SCIENTIFIQUES MEDICALES ELSEVIER, 23 RUE LINOIS, 75724 PARIS CEDEX 15, FRANCE
Language: English Document Type: ARTICLE
Abstract: The rate of protein synthesis in mammals is largely regulated by phosphorylation of the alpha -subunit of eukaryotic initiation factor 2 (eIF2) that is modulated by the cellular glycoprotein, p67, due to its protection of eIF2 alpha phosphorylation (POEP) activity. At the N-terminus of p67, there are three unique domains, and at the C-terminus there is a conserved an-nino acid sequence. To analyze the importance of these domains, C-terminal deletion mutants of rat p67 were expressed constitutively in KRC-7 cells. In these cells, the phosphorylation level of the alpha -subunit of eIF2 was determined, and it was found that expression of the 1-97 amino acid segment of rat p67 increases POEP activity in vivo, and induces the endogenous levels of p67. These cells also show increased growth rate, and efficient translation of chloramphenicol acetyltransferase and beta -galactosidase reporter genes. At the N-terminus of p67, there are two unique domains: a lysine-rich domain I with the sequence,KKKRRKKKK44, and an acidic residue-rich domain with the sequence (77)EEKEKDDDDEDGDGD(91). Substitution of lysine-rich domain I with (36)NMKSGNKTQ(44) in rat recombinant p67 resulted in the inhibition of its POEP activity, and substitution of the acidic residue-rich domain with (77)QNIQKALEPEAGDGA(91), resulted in no inhibition of POEP activity in KRC-7 cells. Taken together, our data suggest that protection of translation initiation factor eIF2 phosphorylation correlates with eIF2-associated glycoprotein p67 levels and requires the lysine-rich domain I of p67. (C) 2001 Societe francaise de biochimie et biologic moleculaire/Editions scientifiques et medicales Elsevier SAS. All rights reserved.

, 2001
...Abstract: conserved an-nino acid sequence. To analyze the importance of these domains, C-terminal deletion mutants of rat p67 were expressed constitutively in KRC-7 cells. In these cells, the phosphorylation...

...the sequence,KKKRRKKKK44, and an acidic residue-rich domain with the sequence (77)EEKEKDDDDEDGDGD(91). Substitution of lysine-rich domain I with (36)NMKSGNKTQ(44) in rat recombinant p67 resulted in the inhibition of its POEP activity, and substitution of the acidic residue-rich domain with (77)QNIQKALEPEAGDGA(91), resulted in no inhibition of...
...Identifiers--PROTEIN-SYNTHESIS INHIBITION; FACTOR 2-ASSOCIATED PROTEIN; METHIONINE AMINOPEPTIDASE; SACCHAROMYCES-CEREVISIAE; 67-KDA POLYPEPTIDE; BETA-SUBUNIT; RETICULOCYTE LYSATE; MOLECULAR-CLONING; ESCHERICHIA-COLI; VIRAL-INFECTION

9/3,K,AB/15 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

08990614 Genuine Article#: 353CR Number of References: 45
Title: An angiogenesis inhibitor: TNP-470
Author(s): Bailly C (REPRINT) ; Lansiaux A
Corporate Source: CTR OSCAR LAMBRET,LAB PHARMACOL ANTITUMORALE, PL VERDUN/F-59045 LILLE//FRANCE/ (REPRINT); INSERM,U524/F-59045 LILLE//FRANCE/
Journal: BULLETIN DU CANCER, 2000, V87, N6 (JUN), P449-454
ISSN: 0007-4551 Publication date: 20000600
Publisher: JOHN LIBBEY EUROTEXT LTD, 127 AVE DE LA REPUBLIQUE, 92120 MONTROUGE, FRANCE
Language: French Document Type: EDITORIAL MATERIAL

, 2000
...Identifiers--BEARING VX-2 CARCINOMA; HUMAN COLON-CANCER; TUMOR-GROWTH; PHASE-I; METHIONINE AMINOPEPTIDASE-2; CHEMICAL MODIFICATION; ENDOTHELIAL-CELLS; SYNTHETIC ANALOGS; LIVER METASTASIS; HUMAN BREAST

9/3,K,AB/16 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

08248404 Genuine Article#: 262RK Number of References: 23
Title: Rabbit kidney aminopeptidases: purification and some properties (ABSTRACT AVAILABLE)
Author(s): Oliveira SM; Freitas JO; Alves KB (REPRINT)
Corporate Source: UNIFESP,ESCOLA PAULISTA MED, DEPT BIOCHEM, CAIXA POSTAL 20372, RUA 3 DE MAIO 100/BR-04044020 SAO PAULO//BRAZIL/ (REPRINT); UNIFESP,ESCOLA PAULISTA MED, DEPT BIOCHEM/BR-04044020 SAO PAULO//BRAZIL/
Journal: IMMUNOPHARMACOLOGY, 1999, V45, N1-3 (DEC), P215-221
ISSN: 0162-3109 Publication date: 19991200
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS
Language: English Document Type: ARTICLE
Abstract: Aminopeptidases (EC.3.4.11...) are widely distributed in nature and have medical and biological importance due to their function in the modification and degradation of protein. Two aminopeptidases were purified from rabbit kidney homogenate by ion exchange and gel filtration chromatography columns, using aminoacyl of

beta-naphthylamides and p-nitroanilides as substrates. The enzymes' homogeneity was assured by SDS-PAGE. The first enzyme (P-1) has an optimum of pH 7.0, a molecular mass of 70 kDa, best catalytical efficiency for methionyl-beta-naphthylamide, is 70% inhibited by 0.5 mM Zn2+ and Co2+ ions, 3.33 mM sodium hydrocortisone succinate and 0.08 mM p-hydroxymercuribenzoate, and is little or not inhibited by EDTA, amino acids, p-nitroaniline, beta-naphthylamine, deoxicholate, bestatin and puromycin. The second enzyme (P-2) has an optimum of pH 7.0, a molecular mass of 54 kDa, best catalytical efficiency for Leu-beta-naphthylamide, is inhibited by 0.5 mM ions Zn2+ (45%), 0.02 mM EDTA (94%) 0.08 mM p-hydroxymercuribenzoate (70%), 3.33 mM beta-ME (13%), 1.33 mM p-nitroaniline (40%), 1.33 mM beta-naphthylamine (17%), 1.33 mM sodium deoxicholate (96%), 3.33 mM sodium hydrocortisone succinate (60%), and is 30% activated by 0.5 mM Co2+ ions. Puromycin and bestatin are competitive inhibitors with K-i values in 10(-6) and 10(-7) M order, respectively. P-1 is a methionine aminopeptidase, while P-2 is a leucine aminopeptidase. (C) 1999 Elsevier Science B.V. All rights reserved.

, 1999
...Abstract: distributed in nature and have medical and biological importance due to their function in the modification and degradation of protein. Two aminopeptidases were purified from rabbit kidney homogenate by ion exchange...

...i values in 10(-6) and 10(-7) M order, respectively. P-1 is a methionine aminopeptidase, while P-2 is a leucine aminopeptidase. (C) 1999 Elsevier Science B.V. All rights reserved.

9/3,K,AB/17 (Item 4 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

05038639 Genuine Article#: TL421 Number of References: 31
Title: AMINO-TERMINAL PROTEIN PROCESSING IN SACCHAROMYCES-CEREVISIAE IS AN ESSENTIAL FUNCTION THAT REQUIRES 2 DISTINCT METHIONINE AMINOPEPTIDASES (Abstract Available)
Author(s): LI X; CHANG YH
Corporate Source: ST LOUIS UNIV,SCH MED,EDWARD A DOISY DEPT BIOCHEM & MOLEC BIOL,1402 S GRAND BLVD/ST LOUIS//MO/63104
Journal: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, 1995, V92, N26 (DEC 19), P12357-12361
ISSN: 0027-8424
Language: ENGLISH Document Type: ARTICLE
Abstract: We previously characterized a methionine aminopeptidase (EC 3.4.11.18; Met-AP1; also called peptidase M) in Saccharomyces cerevisiae, which differs from its prokaryotic homologues in that it (i) contains an N-terminal zinc-finger domain and (ii) does not produce lethality when disrupted, although it does slow growth dramatically; it is encoded by a gene called MAP1. Here we describe a second methionine aminopeptidase (Met-AP2) in S. cerevisiae, encoded by MAP2, which was cloned as a suppressor of the slow-growth phenotype of the map1 null strain. The DNA sequence of MAP2 encodes a protein of 421 amino acids that shows 22% identity with the sequence of yeast Met-AP1. Surprisingly, comparison with sequences in the GenBank data base showed that the product of MAP2 has even greater homology (55% identity) with rat p(67), which was characterized as an initiation factor 2-associated protein but not yet shown to have Met-AP activity. Transformants of map1 null cells expressing MAP2 in a high-copy-number plasmid contained 3-to 12-fold increases in Met-AP activity on different peptide substrates. The epitope-tagged suppressor gene product was purified by immunoaffinity chromatography and shown to contain Met-AP activity; To evaluate the physiological significance of Met-AP2, the MAP2 gene was

deleted from wild-type and map1 null yeast strains. The map2 null strain, like the map1 null strain, is viable but with a slower growth rate. The map1, map2 double-null strains are nonviable. Thus, removal of N-terminal methionine is an essential function in yeast, as in prokaryotes, but yeast require two methionine aminopeptidases to provide the essential function which can only be partially provided by Met-AP1 or Met-AP2 alone.

, 1995
...Identifiers--ESCHERICHIA-COLI; SALMONELLA-TYPHIMURIUM; YEAST; GENE; SPECIFICITIES; MUTATIONS; CLONING; BINDING

9/3,K,AB/18 (Item 1 from file: 340)
DIALOG(R)File 340:CLAIMS(R)/US Patent

Dialog Acc No: 10034443 IFI Acc No: 2001-0034455 IFI Acc No: 2001-0008976
Document Type: C
TYPE 2 METHIONINE AMINOPEPTIDASE (METAP2)
INHIBITORS AND USES THEREOF; ANTI-ANGIOGENIC/IMMUNOSUPPRESSIVE; MEDICAL DIAGNOSIS
Inventors: Griffith Eric C (US); Liu Jun O (US); Su Zhuang (US)
Assignee: Unassigned Or Assigned To Individual
Assignee Code: 68000
Publication (No,Date), Applic (No,Date):
US 20010034455 20011025 US 2001813555 20010321
Publication Kind: A1
Division Pub(No),Applic(No,Date): US 6207704 US 9893448 19980608
Priority Applic(No,Date): US 2001813555 20010321; US 9893448 19980608
Provisional Applic(No,Date): US 60-49159 19970609

Abstract: Novel compounds that are anti-angiogenic or immunosuppressive are described. Also described are methods for determining if an animal is at risk for a disease involving abnormal angiogenesis or an immune reaction resulting in pathology comprising evaluating an aspect of MetAP2 metabolism or structure; methods for identifying agents that are anti-angiogenic or immunosuppressive comprising evaluating the effect of the agent on an aspect of MetAP2 metabolism; methods for treating a cell having an abnormality in metabolism or structure of MetAP2; and methods for treating abnormal angiogenesis or an immune reaction which results in pathology in an animal. Pharmaceutical compositions are also provided.

TYPE 2 METHIONINE AMINOPEPTIDASE (METAP2)
INHIBITORS AND USES THEREOF...
Publication (No,Date), Applic (No,Date):
...20011025
Publication Kind: A1

Abstract: ...involving abnormal angiogenesis or an immune reaction resulting in pathology comprising evaluating an aspect of MetAP2 metabolism or structure; methods for identifying agents that are anti-angiogenic or immunosuppressive comprising evaluating the effect of the agent on an aspect of MetAP2 metabolism; methods for treating a cell having an abnormality in metabolism or structure of MetAP2; and methods for treating abnormal angiogenesis or an immune reaction which results in pathology in...

Exemplary Claim: ...P1, P2 and P3 can be the same or different and are each an optionally substituted hydrocarbon or heterocyclic group and X- is a counter anion; R1, R2, R3, R4, R5...

...alkyl, aryl, halogen, hydroxyl, alkoxy, carbamoyl, carbonyldioxyl, thiohydroxyl, amino, alkylamino, dialkylamino, ureido, lower alkoxy, a substituted alkanoyl group, a cyclic or aromatic group which can be optionally substituted, a heterocyclic or aromatic heterocyclic group which can be optionally substituted, a substituted aryl or aroyl group having at least one substituent selected from the group consisting of...

...and alkylthioether; R7 is hydrogen or an hydroxy group; and R8is (1) hydrogen or a substituted alkyl, allyl or alkyne group; or (2) a substituted alkoxyl or thioalkoxyl group, or methylene or ethylene alkoxyl or thioalkoxyl group, wherein the methylene or ethylene can be optionally substituted; (3) an aroyl group which can be optionally substituted with at least one substituent selected from the group consisting of alkyl, amino, alkylamino, dialkylamino...

...alkoxyl, cyano, amido, carbamoyl, thiocarbamoyl, carbonyldioxyl, carboxylic acid, carboxyl ester, carboxyl salt, alkyl or dialkylcarbamoyl, substituted ureido, vinyl, cyclic or aromatic cyclic groups which can be optionally substituted, or a heterocyclic or aromatic heterocyclic group which can be optionally substituted; or (4) an aryl group which can be optionally substituted with at least one substituent selected from the group consisting of alkyl, amino, alkylamino, dialkylamino...

...alkoxy, cyano, amido, carbamoyl, thiocarbamoyl, carbonyldioxyl, carboxylic acid, carboxyl ester, carboxyl salt, alkyl or dialkylcarbamoyl, substituted ureido, vinyl, cyclic or aromatic cyclic groups which can be optionally substituted, or a heterocyclic or aromatic heterocyclic group which can be optionally substituted; or (5) an amino, alkylamino, dialkylamino, halogen, hydroxyl, cyano, amido, carbamoyl, thiocarbamoyl, carbonyldioxyl, carboxyl, alkyl, dialkylcarbamoyl, ureido, vinyl, cyclic or aromatic cyclic groups which can be optionally substituted, a heterocyclic or aromatic heterocyclic group which can be optionally substituted , carboxylic acid, carboxyl ester, carboxyl salt; or (6) 2methyl-1 -propenyl or an isobutyl group which can be optionally substituted with hydroxyl, carbamoyl, carbonyldioxyl, thiohydroxyl, amino, alkylamino, dialkylamino, ureido, alky, lower alkoxy, a substituted alkanoyl group, a cyclic or aromatic cyclic group which can be optionally substituted, a heterocyclic or aromatic heterocyclic group which can be optionally substituted, a substituted aryl or aroyl group having at least one substituent selected from the group consisting of...

...a methylene or ethylene substituent, or the corresponding amide or ester, which can be optionally substituted; or (8) an alkoxycarbonyl or phenoxycarbonyl group with or without a methylene or ethylene substituent, which can be optionally substituted.

Non-exemplary Claims: ...from the compounds of claim 1, said agent being capable of altering an aspect of MetAP2 metabolism or structure in said animal so as to result in treatment of said abnormal...

...P1, P2 and P3 can be the same or different and are each an optionally substituted hydrocarbon or heterocyclic group and X- is a counter anion; R1, R2, R3, R4, R5...

...alkyl, aryl, halogen, hydroxyl, alkoxy, carbamoyl, carbonyldioxyl, thiohydroxyl, amino, alkylamino, dialkylamino, ureido, lower alkoxy, a substituted alkanoyl group, a cyclic or aromatic group which can be optionally substituted, a heterocyclic or aromatic heterocyclic group which can be optionally substituted, a substituted aryl or aroyl group having at least one substituent selected from the

group consisting of...

...and alkylthioether; R7 is hydrogen or an hydroxy group; and R8is (1) hydrogen or a substituted alkyl, allyl or alkyne group; (2) a substituted alkoxyl or thioalkoxyl group, or methylene or ethylene alkoxyl or thioalkoxyl group, wherein the methylene or ethylene can be optionally substituted; (3) an aroyl group which can be optionally substituted with at least one substituent selected from the group consisting of alkyl, amino, alkylamino, dialkylamino...

...alkoxyl, cyano, amido, carbamoyl, thiocarbamoyl, carbonyldioxyl, carboxylic acid, carboxyl ester, carboxyl salt, alkyl or dialkylcarbamoyl, substituted ureido, vinyl, cyclic or aromatic cyclic groups which can be optionally substituted, or a heterocyclic or aromatic heterocyclic group which can be optionally substituted; or (4) an aryl group which can be optionally substituted with at least one substituent selected from the group consisting of alkyl, amino, alkylamino, dialkylamino...

...alkoxy, cyano, amido, carbamoyl, thiocarbamoyl, carbonyldioxyl, carboxylic acid, carboxyl ester, carboxyl salt, alkyl or dialkylcarbamoyl, substituted ureido, vinyl, cyclic or aromatic cyclic groups which can be optionally substituted, or a heterocyclic or aromatic heterocyclic group which can be optionally substituted; or (5) an amino, alkylamino, dialkylamino, halogen, hydroxyl, cyano, amido, carbamoyl, thiocarbamoyl, carbonyldioxyl, carboxyl, alkyl, dialkylcarbamoyl, ureido, vinyl, cyclic or aromatic cyclic groups which can be optionally substituted, a heterocyclic or aromatic heterocyclic group which can be optionally substituted , carboxylic acid, carboxyl ester, carboxyl salt; or (6) 2-methyl-1-propenyl or an isobutyl group which can be optionally substituted with hydroxyl, carbamoyl, carbonyldioxyl, thiohydroxyl, amino, alkylamino, dialkylamino, ureido, alky, lower alkoxy, a substituted alkanoyl group, a cyclic or aromatic cyclic group which can be optionally substituted, a heterocyclic or aromatic heterocyclic group which can be optionally substituted, a substituted aryl or aroyl group having at least one substituent selected from the group consisting of...

...a methylene or ethylene substituent, or the corresponding amide or ester, which can be optionally substituted; or (8) an alkoxycarbonyl or phenoxycarbonyl group with or without a methylene or ethylene substituent, which can be optionally substituted.
...

...selected from compounds of claim 1, said agent being capable of altering an aspect of MetAP2 metabolism or structure in said animal so as to result in treatment of said immune...P1, P2 and P3 can be the same or different and are each an optionally substituted hydrocarbon or heterocyclic group and X- is a counter anion; R1, R2, R3, R4, R5...

...alkyl, aryl, halogen, hydroxyl, alkoxy, carbamoyl, carbonyldioxyl, thiohydroxyl, amino, alkylamino, dialkylamino, ureido, lower alkoxy, a substituted alkanoyl group, a cyclic or aromatic group which can be optionally substituted, a heterocyclic or aromatic heterocyclic group which can be optionally substituted, a substituted aryl or aroyl group having at least one substituent selected from the group consisting of...

...and alkylthioether; R7 is hydrogen or an hydroxy group; and R8is (1) hydrogen or a substituted alkyl, allyl or alkyne group; (2) a substituted alkoxyl or thioalkoxyl group, or methylene or ethylene alkoxyl or thioalkoxyl group, wherein the methylene or ethylene can be

optionally substituted; (3) an aroyl group which can be optionally substituted with at least one substituent selected from the group consisting of alkyl, amino, alkylamino, dialkylamino...

...alkoxyl, cyano, amido, carbamoyl, thiocarbamoyl, carbonyldioxyl, carboxylic acid, carboxyl ester, carboxyl salt, alkyl or dialkylcarbamoyl, substituted ureido, vinyl, cyclic or aromatic cyclic groups which can be optionally substituted, or a heterocyclic or aromatic heterocyclic group which can be optionally substituted; or (4) an aryl group which can be optionally substituted with at least one substituent selected from the group consisting of alkyl, amino, alkylamino, dialkylamino...

...alkoxy, cyano, amido, carbamoyl, thiocarbamoyl, carbonyldioxyl, carboxylic acid, carboxyl ester, carboxyl salt, alkyl or dialkylcarbamoyl, substituted ureido, vinyl, cyclic or aromatic cyclic groups which can be optionally substituted, or a heterocyclic or aromatic heterocyclic group which can be optionally substituted; or (5) an amino, alkylamino, dialkylamino, halogen, hydroxyl, cyano, amido, carbamoyl, thiocarbamoyl, carbonyldioxyl, carboxyl, alkyl, dialkylcarbamoyl, ureido, vinyl, cyclic or aromatic cyclic groups which can be optionally substituted, a heterocyclic or aromatic heterocyclic group which can be optionally substituted , carboxylic acid, carboxyl ester, carboxyl salt; or (6) 2-methyl-1-propenyl or an isobutyl group which can be optionally substituted with hydroxyl, carbamoyl, carbonyldioxyl, thiohydroxyl, amino, alkylamino, dialkylamino, ureido, alky, lower alkoxy, a substituted alkanoyl group, a cyclic or aromatic cyclic group which can be optionally substituted, a heterocyclic or aromatic heterocyclic group which can be optionally substituted, a substituted aryl or aroyl group having at least one substituent selected from the group consisting of...

...a methylene or ethylene substituent, or the corresponding amide or ester, which can be optionally substituted; or (8) an alkoxycarbonyl or phenoxycarbonyl group with or without a methylene or ethylene substituent, which can be optionally substituted.

9/3,K,AB/19 (Item 2 from file: 340)
DIALOG(R)File 340:CLAIMS(R)/US Patent

Dialog Acc No: 3522280 IFI Acc No: 0121441
Document Type: C
SUBSTITUTED BETA-AMINO ACID INHIBITORS OF METHIONINE AMINOPEPTIDASE-2; ANGIOGENESIS INHIBITORS, ANTITUMOR AGENTS, DIABETIC RETINOPATHY, AND ANTIINFLAMMATORY AGENTS
Inventors: Craig Richard A (US); Henkin Jack (US); Kawai Megumi (US); Lynch Linda M (US); Patel Jyoti (US); Sheppard George S (US); Wang Jieyi (US)
Assignee: Abbott Laboratories
Assignee Code: 00152
Publication (No,Date), Applic (No,Date):
US 6242494 20010605 US 99303807 19990430
Publication Kind: B
Calculated Expiration: 20190430
Priority Applic(No,Date): US 99303807 19990430
Provisional Applic(No,Date): US 60-83877 19980501

Abstract: A class of substituted b-amino acids are potent inhibitor of methionine aminopeptidase type 2 (MetAP2) and are thus useful in inhibiting angiogenesis and disease conditions which depend upon angiogenesis for their development such as diabetic retinopathy, tumor growth, and conditions of inflammation. Pharmaceutical

compounds containing the compounds and methods of inhibiting methionine aminopeptidase-2, and angiogenesis are also disclosed.

SUBSTITUTED BETA-AMINO ACID INHIBITORS OF METHIONINE AMINOPEPTIDASE-2;
Publication (No,Date), Applic (No,Date):
...20010605
Publication Kind: B

Abstract: A class of substituted b-amino acids are potent inhibitor of methionine aminopeptidase type 2 (MetAP2) and are thus useful in inhibiting angiogenesis and disease conditions which depend upon angiogenesis for...

...tumor growth, and conditions of inflammation. Pharmaceutical compounds containing the compounds and methods of inhibiting methionine aminopeptidase-2, and angiogenesis are also disclosed.

Exemplary Claim: ...of (1) hydrogen, (2) alkyl, (3) carboxaldehyde, (4) alkanoyl, where the alkanoyl can be optionally substituted with hydroxyl, and (5) --(CH2)n CO2 R4, where n is 0-6, and R4...

...d) (cycloalkyl)alkyl, (e) aryl, and (f) arylalkyl, where (c) and (d) can be optionally substituted with 1, 2, or 3 substituents independently selected from the group consisting of (i) alkyl, (ii) alkoxy, and (iii) aryl, and where (e) and (f) can be optionally substituted with 1, 2, or 3 substituents independently selected from the group consisting of (i) alkyl...

...and R6' are independently selected from the group consisting of (1) hydrogen, (2) alkyl optionally substituted with alkoxy, (3) aryl, (4) arylalkyl, and (5') a nitrogen-protecting group, (xvi) --SO2 NR6...

...alkyl, (b) cycloalkyl, (c) (cycloalkyl)alkyl, and (d) benzyl, where the benzyl can be optionally substituted with 1, 2, or 3 substituents independently selected from the group consisting of (i) alkyl...

...from the group consisting of (a) hydrogen, (b) alkyl, where the alkyl can be optionally substituted with 1, 2, 3, or 4 substituents independently selected from the group consisting of (i...

...defined above, (xvi) aryl, and (xvii) hydroxy, (c) cycloalkyl, where the cycloalkyl can be optionally substituted with 1, 2, or 3 substituents indepdently selected from the group consisting of (i) alkyl, (ii) halo, (iii) oxo, and (iv) aryl, (d) aryl, where the aryl can be optionally substituted with 1, 2, or 3 substituents independently selected from the group consisting of (i) alkyl...

Non-exemplary Claims: ...of (i) hydrogen (ii) alkyl, and (iii) aryl, where (ii) and (iii) can be optionally substituted with one, two, or three groups independently selected from the group consisting of (1') alkyl...

...of (i) alkyl, (ii) aryl, and (iii) arylalkyl, where (ii) and (iii) can be optionally substituted with 1, 2, or 3 groups independently selected from the group consisting of (1') alkyl...

9/3,K,AB/20 (Item 3 from file: 340)
DIALOG(R)File 340:CLAIMS(R)/US Patent

Dialog Acc No: 3483617 IFI Acc No: 0111593

Document Type: C
TYPE 2 METHIONINE AMINOPEPTIDASE (METAP2) INHIBITORS AND USES THEREOF; FOR TREATING ABNORMAL ANGIOGENESIS IN AN ANIMAL; FOR TREATING AN IMMUNE REACTION WHICH RESULTS IN PATHOLOGY IN AN ANIMAL; DIAGNOSIS
Inventors: Griffith Eric C (US); Liu Jun O (US); Su Zhuang (US)
Assignee: Massachusetts Institute of Technology
Assignee Code: 52912
Publication (No,Date), Applic (No,Date):
US 6207704 20010327 US 9893448 19980608
Publication Kind: B
Calculated Expiration: 20180608
Priority Applic(No,Date): US 9893448 19980608
Provisional Applic(No,Date): US 60-49159 19970609

Abstract: Novel compounds that are anti-angiogenic or immunosuppressive are described. Also described are methods for determining if an animal is at risk for a disease involving abnormal angiogenesis or an immune reaction resulting in pathology comprising evaluating an aspect of MetAP2 metabolism or structure; methods for identifying agents that are anti-angiogenic or immunosuppressive comprising evaluating the effect of the agent on an aspect of MetAP2 metabolism; methods for treating a cell having an abnormality in metabolism or structure of MetAP2; and methods for treating abnormal angiogenesis or an immune reaction which results in pathology in an animal. Pharmaceutical compositions are also provided.

TYPE 2 METHIONINE AMINOPEPTIDASE (METAP2) INHIBITORS AND USES THEREOF...
Publication (No,Date), Applic (No,Date):
...20010327
Publication Kind: B

Abstract: ...involving abnormal angiogenesis or an immune reaction resulting in pathology comprising evaluating an aspect of MetAP2 metabolism or structure; methods for identifying agents that are anti-angiogenic or immunosuppressive comprising evaluating the effect of the agent on an aspect of MetAP2 metabolism; methods for treating a cell having an abnormality in metabolism or structure of MetAP2; and methods for treating abnormal angiogenesis or an immune reaction which results in pathology in...

Exemplary Claim: ...P1, P2 and P3 can be the same or different and are each an optionally substituted hydrocarbon or heterocyclic group and X- is a counter anion; R1, R2, R3, R4, R5...

...alkyl, aryl, halogen, hydroxyl, alkoxy, carbamoyl, carbonyldioxyl, thiohydroxyl, amino, alkylamino, dialkylamino, ureido, lower alkoxy, a substituted alkanoyl group, a cyclic or aromatic group which can be optionally substituted, a heterocyclic or aromatic heterocyclic group which can be optionally substituted, a substituted aryl or aroyl group having at least one substituent selected from the group consisting of...

...can be the same or different from each other and are: (1) hydrogen or a substituted alkyl, allyl or alkyne group; (2) a substituted alkoxyl or thioalkoxyl group, or methylene or ethylene alkoxyl or thioalkoxyl group, wherein the methylene or ethylene can be optionally substituted; (3) an aroyl group which can be optionally substituted with at least one substituent selected from the group consisting of alkyl, amino, alkylamino, dialkylamino...

...alkoxyl, cyano, amido, carbamoyl, thiocarbamoyl, carbonyldioxyl,

carboxylic acid, carboxyl ester, carboxyl salt, alkyl or dialkylcarbamoyl, substituted ureido, vinyl, cyclic or aromatic cyclic groups which can be optionally substituted, or a heterocyclic aromatic heterocyclic group which can be optionally substituted; or (4) an aryl group which can be optionally substituted with at least one substituent selected from the group consisting of alkyl, amino, alkylamino, dialkylamino...

...alkoxy, cyano, amido, carbamoyl, thiocarbamoyl, carbonyldioxyl, carboxylic acid, carboxyl ester, carboxyl salt, alkyl or dialkylcarbamoyl, substituted ureido, vinyl, cyclic or aromatic cyclic groups which can be optionally substituted, or a heterocyclic or aromatic heterocyclic group which can be optionally substituted; or (5) an amino, alkylamino, dialkylamino, halogen, hydroxyl, cyano, amido, carbamoyl, thiocarbamtoyl, carbonyldioxyl, carboxyl, alkyl, dialkylcarbamoyl, ureido, vinyl, cyclic or aromatic cyclic groups which can be optionally substituted, a heterocyclic or aromatic heterocyclic group which can be optionally substituted , carboxylic acid, carboxyl ester, carboxyl salt; or (6) 2-methyl-1-propenyl or an isobutyl group which can be optionally substituted with hydroxyl, carbamoyl, carbonyldioxyl, thiohydroxyl, amino, alkylamino, dialkylamino, ureido, alky, lower alkoxy, a substituted alkanoyl group, a cyclic or aromatic cyclic group which can be optionally substituted, a heterocyclic or aromatic heterocyclic group which can be optionally substituted, a substituted aryl or aroyl group having at least one substituent selected from the group consisting of...

...a methylene or ethylene substituent, or the corresponding amide or ester, which can be optionally substituted; or (8) an alkoxycarbonyl or phenoxycarbonyl group with or without a methylene or ethylene substituent, which can be optionally substituted.

Non-exemplary Claims: ...selected from compounds of claim 1, said agent being capable of altering an aspect of MetAP2 metabolism or structure in said animal so as to result in treatment of said abnormal ...

...P1, P2 and P3 can be the same or different and are each an optionally substituted hydrocarbon or heterocyclic group and X- is a counter anion; R1, R2, R3, R4, R5...

...alkyl, aryl, halogen, hydroxyl, alkoxy, carbamoyl, carbonyldioxyl, thiohydroxyl, amino alkylamino, dialkylamino, ureido, lower alkoxy, a substituted alkanoyl group, a cyclic or aromatic group which can be optionally substituted, a heterocyclic or aromatic heterocyclic group which can be optionally substituted, a substituted aryl or aroyl group having at least one substituent selected from the group consisting of...

...can be the same or different from each other and are: (1) hydrogen or a substituted alkyl, allyl or alkyne group; (2) a substituted alkoxyl or thioalkoxyl group, or methylene or ethylene alkoxyl or thioalkoxyl group, wherein the methylene or ethylene can be optionally substituted; (3) an aroyl group which can be optionally substituted with at least one substituent selected from the group consisting of alkyl, amino, alkylamino, dialkylamino...

...alkoxyl, cyano, amido, carbamoyl, thiocarbamoyl, carbonyldioxyl, carboxylic acid, carboxyl ester, carboxyl salt, alkyl or dialkylcarbamoyl, substituted ureido, vinyl, cyclic or aromatic cyclic groups which can be optionally substituted, or a heterocyclic or aromatic heterocyclic group which can be optionally substituted; or (4) an aryl group which can be optionally substituted with at least one substituent selected from the group

consisting of alkyl, amino, alkylamino dialkylamino...
...alkoxy, cyano, amido, carbamoyl, thiocarbamoyl, carbonyldioxyl, carboxylic acid, carboxyl ester, carboxyl salt, alkyl or dialkylcarbamoyl, substituted ureido, vinyl, cyclic or aromatic cyclic groups which can be optionally substituted, or a heterocyclic or aromatic heterocyclic group which can be optionally substituted; or (5) an amino, alkylamino, dialkylamino, halogen, hydroxyl, cyano, amido, carbamoyl, thiocarbamoyl, carbonyldioxyl, carboxyl, alkyl, dialkylcarbamoyl, ureido, vinyl, cyclic or aromatic cyclic groups which can be optionally substituted, a heterocyclic or aromatic heterocyclic group which can be optionally substituted , carboxylic acid, carboxyl ester, carboxyl salt; or (6) 2-methyl-1-propenyl or an isobutyl (group which can be optionally substituted with hydroxyl, carbamoyl, carbonyldioxyl, thiohydroxyl, amino, alkylamino, dialkylamino, ureido, alky, lower alkoxy, a substituted alkanoyl group, a cyclic or aromatic cyclic group which can be optionally substituted, a heterocyclic or aromatic heterocyclic group which can be optionally substituted, a substituted aryl or aroyl group having at least one substituent selected from the group consisting of...a methylene or ethylene substituent, or the corresponding amide or ester, which can be optionally substituted; or (8) an alkoxycarbonyl or phenoxycarbonyl group with or without a methylene or ethylene substituent, which can be optionally substituted.
...

...selected from compounds of claim 1, said agent being capable of altering an aspect of MetAP2 metabolism or structure in said animal so as to result in treatment of said immune...

...P1, P2 and P3 can be the same or different and are each an optionally substituted hydrocarbon or heterocyclic group and X- is a counter anion; R1, R2, R3, R4, R5...

...alkyl, aryl, halogen, hydroxyl, alkoxy, carbamoyl, carbonyldioxyl, thiohydroxyl, amino, alkylamino, dialkylamino, ureido, lower alkoxy a substituted alkanoyl group, a cyclic or aromatic group which can be optionally substituted, a heterocyclic or aromatic heterocyclic group which can be optionally substituted, a substituted aryl or aroyl group having at least one substituent selected from the group consisting of...

...can be the same or different from each other and are: (1) hydrogen or a substituted alkyl, allyl or alkyne group; (2) a substituted alkoxyl or thioalkoxyl group, or methylene or ethylene alkoxyl or thioalkoxyl group, wherein the methylene or ethylene can be optionally substituted; (3) an aroyl group which can be optionally substituted with at least one substituent selected from the group consisting of alkyl, amino, alkylamino, dialkylamino...

...alkoxyl, cyano, amido, carbamoyl thiocarbamoyl carbonyldioxyl, carboxylic acid carboxyl ester, carboxyl salt, alkyl or dialkylcarbamoyl, substituted ureido, vinyl, cyclic or aromatic cyclic groups which can be optionally substituted, or a heterocyclic or aromatic heterocyclic group which can be optionally substituted; or (4) an aryl group which can be optionally substituted with at least one ...alkoxy, cyano, amido, carbamoyl, thiocarbamoyl, carbonyldioxyl, carboxylic acid, carboxyl ester, carboxyl salt, alkyl or dialkylcarbamoyl, substituted ureido, vinyl, cyclic or aromatic cyclic groups which can be optionally substituted, or a heterocyclic or aromatic heterocyclic group which can be optionally substituted; or (5) an amino, alkylamino, dialkylamino, halogen, hydroxyl, cyano, amido, carbamoyl, thiocarbamoyl, carbonyldioxyl, carboxyl, alkyl, dialkylcarbamoyl, ureido, vinyl, cyclic or aromatic

cyclic groups which can be optionally substituted, a heterocyclic or aromatic heterocyclic group which can be optionally substituted , carboxylic acid, carboxyl ester, carboxyl salt; or (6) 2-methyl-1-propenyl or an isobutyl group which can be optionally substituted with hydroxyl, carbamoyl, carbonyldioxyl, thiohydroxyl, amino, alkylamino, dialkylamino, ureido, alky, lower alkoxy, a substituted alkanoyl group, a cyclic or aromatic cyclic group which can be optionally substituted, a heterocyclic or aromatic heterocyclic group which can be optionally substituted, a substituted aryl or aroyl group having at least one substituent selected from the group consisting of...

...a methylene or ethylene substituent, or the corresponding amide or ester, which can be optionally substituted; or (8) an alkoxycarbonyl or phenoxycarbonyl group with or without a methylene or ethylene substituent, which can be optionally substituted.

9/3,K,AB/21 (Item 4 from file: 340)
DIALOG(R)File 340:CLAIMS(R)/US Patent

Dialog Acc No: 3405083 IFI Acc No: 0035129
Document Type: C
ANTISENSE INHIBITION OF METHIONINE AMINOPEPTIDASE 2 EXPRESSION; OLIGONUCLEOTIDES WHICH TARGET PREFERENTIAL NUCLEOTIDE SEQUENCES FOR THE SUPPRESSION ON EXPRESSION OF HUMAN ENZYME; FOR TREATMENT OF SKIN DISORDERS; ANTICARCINOGENIC AGENTS; ANTIINFLAMMATORY AGENTS
Inventors: Monia Brett P (US); Wyatt Jacqueline (US)
Assignee: ISIS Pharmaceuticals Inc
Assignee Code: 28846
Publication (No,Date), Applic (No,Date):
US 6136604 20001024 US 99428584 19991027
Publication Kind: A
Calculated Expiration: 20191027
(Cited in 001 later patents)
Priority Applic(No,Date): US 99428584 19991027

Abstract: Antisense compounds, compositions and methods are provided for modulating the expression of methionine aminopeptidase 2. The compositions comprise antisense compounds, particularly antisense oligonucleotides, targeted to nucleic acids encoding methionine aminopeptidase 2. Methods of using these compounds for modulation of methionine aminopeptidase 2 expression and for treatment of diseases associated with expression of methionine aminopeptidase 2 are provided.

ANTISENSE INHIBITION OF METHIONINE AMINOPEPTIDASE 2 EXPRESSION...
Publication (No,Date), Applic (No,Date):
...20001024

Abstract: Antisense compounds, compositions and methods are provided for modulating the expression of methionine aminopeptidase 2. The compositions comprise antisense compounds, particularly antisense oligonucleotides, targeted to nucleic acids encoding methionine aminopeptidase 2. Methods of using these compounds for modulation of methionine aminopeptidase 2 expression and for treatment of diseases associated with expression of methionine aminopeptidase 2 are provided.

Exemplary Claim: ...of a 3'-untranslated region, or nucleobases 69-1414 of a coding region of human methionine aminopeptidase 2

(SEQ ID NO:3), wherein said antisense compound specifically hybridizes with and inhibits the expression of human methionine aminopeptidase 2.

Non-exemplary Claims: ...3. The antisense compound of claim 2 wherein the antisense oligonucleotide comprises at least one modified internucleoside linkage...

...4. The antisense compound of claim 3 wherein the modified internucleoside linkage is a phosphorothioate linkage...

...5. The antisense compound of claim 2 wherein the antisense oligonucleotide comprises at least one modified sugar moiety...

...6. The antisense compound of claim 5 wherein the modified sugar moiety is a 2'-O-methoxyethyl sugar moiety...

...7. The antisense compound of claim 2 wherein the antisense oligonucleotide comprises at least one modified nucleobase...

...8. The antisense compound of claim 7 wherein the modified nucleobase is a 5-methylcytosine...

...13. A method of inhibiting the expression of human methionine aminopeptidase 2 in human cells or tissues comprising contacting said cells or tissues in vitro with the antisense compound of claim 1 so that expression of human methionine aminopeptidase 2 is inhibited...

...80, 81, 82, 83, 84, 85, 86 or 87 which inhibits the expression of human methionine aminopeptidase 2.

...

...16. The antisense compound of claim 15 wherein the antisense oligonucleotide comprises at least one modified internucleoside linkage...

...17. The antisense compound of claim 16 wherein the modified internucleoside linkage is a phosphorothioate linkage...

...18. The antisense compound of claim 15 wherein the antisense oligonucleotide comprises at least one modified sugar moiety...

...19. The antisense compound of claim 18 wherein the modified sugar moiety is a 2'-O-methoxyethyl sugar moiety...

...20. The antisense compound of claim 15 wherein the antisense oligonucleotide comprises at least one modified nucleobase...

...21. The antisense compound of claim 20 wherein the modified nucleobase is a 5-methylcytosine...

...23. A method of inhibiting the expression of human methionine aminopeptidase 2 in human cells or tissues comprising contacting said cells or tissues in vitro with the antisense compound of claim 14 so that expression of human methionine aminopeptidase 2 is inhibited.

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? b 340
       31jan03 15:33:36 User231882 Session D1134.2
            $0.00    0.073 DialUnits File410
     $0.00  Estimated cost File410
     $0.05  TELNET
     $0.05  Estimated cost this search
     $0.05  Estimated total session cost   0.310 DialUnits

File 340:CLAIMS(R)/US Patent  1950-03/Jan 28
       (c) 2003 IFI/CLAIMS(R)
*File 340: The Claims U.S. Patent databases have been reloaded.
 HELP NEWS340 & HELP ALERTS340 for search, display & Alert info.

      Set  Items  Description
      ---  -----  -----------
? s adenovirus(5n)vector
            1128  ADENOVIRUS
           35467  VECTOR
      S1     339  ADENOVIRUS(5N)VECTOR
? s cmv(5n)promoter
             526  CMV
           14744  PROMOTER
      S2     222  CMV(5N)PROMOTER
? s s1 and s2
             339  S1
             222  S2
      S3      23  S1 AND S2
? s s3 and py<2001
              23  S3
         3460184  PY<2001
      S4       7  S3 AND PY<2001
? t s4/3,k,ab/1-7

 4/3,K,AB/1
DIALOG(R)File 340:CLAIMS(R)/US Patent
(c) 2003 IFI/CLAIMS(R). All rts. reserv.

Dialog Acc No: 3412639  IFI Acc No: 0036961
Document Type: C
TUMOR REGRESSION BY ADENOVIRUS EXPRESSION OF WILD-TYPE P53; ADMINISTERING
GENETIC ENGINEERED CELLS WHICH INDUCE CELL DEATH IN HUMAN TUMOR CELLS
Inventors: Roth Jack A (US); Zhang Wei-Wei (US)
Assignee: Texas, University of System
Assignee Code: 83960
Publication (No,Date), Applic (No,Date):
US 6143290     20001107 US 94224232    19940407
Publication Kind:  A
Calculated Expiration: 20171107
Document Type: CERTIFICATE OF CORRECTION  Certificate of Correction Date:
    20010529
Cont.-in-part Pub(No),Applic(No,Date): US 6017524                US
92960513     19921013
Division Pub(No),Applic(No,Date):                              US 93145826
19931029
Priority Applic(No,Date): US 94224232    19940407; US 92960513    19921013;
US 93145826    19931029

Abstract: Described are simplified and efficient methods for preparing
recombinant adenovirus using liposome-mediated cotransfection and the
direct observation of a cytopathic effect (CPE) in the transfected cells.
Also disclosed are compositions and methods involving novel p53 adenovirus
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constructs, including methods for restoring p53 function and tumor suppression in cells and animals having abnormal p53.

Publication (No,Date), Applic (No,Date):
...**20001107**

Exemplary Claim: ...cells comprising administering directly to a tumor comprised of cells which lack functional p53, an **adenovirus vector** which does not express functional E1B, wherein the vector further comprises and expresses a DNA...
Non-exemplary Claims: ...10. The method of claim 1, wherein the **vector** comprises **adenovirus** type-5 sequences...

...14. The method of claim 1, wherein the **vector** is a replication-defective **adenovirus**.
...29. The method of claim 1, wherein about 103 to 5X1012 **adenovirus vector** particles are administered...

...30. The method of claim 29, wherein about 1010 to 5X1012 **adenovirus vector** particles are administered...

...31. The method of claim 30, wherein about 1010 **adenovirus vector** particles are administered32. The method of claim 30, wherein about 5X1012 **adenovirus vector** particles are administered...cells comprising administering directly to a tumor comprised of cells which lack functional p53, an **adenovirus vector** which does not express functional E1B, wherein the vector further comprises and expresses a DNA sequence encoding wild-type p53 operably linked to the **CMV** IE **promoter**, and wherein sufficient wild-type p53 is expressed in the tumor cells to induce cell ...
...58. The method of claim 49, wherein the **vector** comprises **adenovirus** type-5 sequences61. The method of claim 49, wherein the **vector** is a replication-defective **adenovirus**.
...

...75. The method of claim 49, wherein about 103 to 5X1012 **adenovirus vector** particles are administered76. The method of claim 75, wherein about 1010 to 5X1012 **adenovirus vector** particles are administered...

...77. The method of claim 76, wherein about 1010 **adenovirus vector** particles are administered...

...78. The method of claim 76, wherein about 5X1012 **adenovirus vector** particles are administered...

4/3,K,AB/2
DIALOG(R)File 340:CLAIMS(R)/US Patent

Dialog Acc No: 3376616 IFI Acc No: 0027693
Document Type: C
DIMINISHING VIRAL GENE EXPRESSION BY PROMOTER REPLACEMENT; PRODUCING INFECTIOUS, CONDITIONALLY REPLICATION-DEFECTIVE ADENOVIRUS PARTICLE BY CULTURING CELL WITH HETEROLOGOUS GENE CODING A FACTOR THAT INDUCES A PROMOTER WITH **ADENOVIRUS VECTOR** HAVING ESSENTIAL VIRAL GENE UNDER CONTROL OF PROMOTER
Inventors: Fang Bingliang (US); Roth Jack A (US)
Assignee: Texas, University of System
Assignee Code: 83960
Publication (No,Date), Applic (No,Date):

US 6110744 **20000829** US 97968014 19971112
Publication Kind: A
Calculated Expiration: 20171112
Document Type: CERTIFICATE OF CORRECTION Certificate of Correction Date: 20010515
Priority Applic(No,Date): US 97968014 19971112
Provisional Applic(No,Date): US 60-30675 19961113

Abstract: The present invention provides viral vectors that have been engineered to contain a synthetic promoter that controls at least one essential gene. The synthetic promoter is induced by a specific gene product not normally produced in the cells in which the viral vector is to be transferred. The vectors are propagated in producer or helper cells that express the inducing factor, thereby permitting the virus to replicate to high titer. The lack of the inducing factor in the target cells precludes viral replication, however, meaning that no vector toxicity or immunogenicity arises. Where the virus carries a gene of interest, this should provide for higher level expression for longer periods of time than with current vectors. Methods for making the vectors, helper cells, and their use in protein production, vaccines and gene therapy are disclosed.

...PARTICLE BY CULTURING CELL WITH HETEROLOGOUS GENE CODING A FACTOR THAT INDUCES A PROMOTER WITH **ADENOVIRUS VECTOR** HAVING ESSENTIAL VIRAL GENE UNDER CONTROL OF PROMOTER
Publication (No,Date), Applic (No,Date):
...**20000829**

Exemplary Claim: ...induces a promoter active in eukaryotic cells; (b) contacting said cell with a conditionally replicationdefective **adenovirus vector**, said **adenovirus vector** comprising at least one essential viral gene or gene element under the control of a...

...by said first factor; (c) culturing said cell under conditions permitting the uptake of said **adenovirus vector** by, and replication in, said cell; and (d) harvesting said infectious, conditionally replicationdefective adenovirus particle...
Non-exemplary Claims: ...1, wherein said cell further comprises an essential viral gene or gene element and said **adenovirus vector** lacks a functional copy of said essential viral gene or gene element...

...claim 2, wherein at least one viral gene or gene element is deleted from the **adenovirus vector**.
...

...claim 6, wherein at least two viral genes or gene elements are deleted from the **adenovirus vector**.
...

...10. The method of claim 6, wherein the **adenovirus vector** comprises an E2 gene under the control of an inducible promoter...

...11. The method of claim 10, wherein the **adenovirus vector** comprises an E5 gene under the control of an inducible promoter...

...12. The method of claim 1, wherein said **adenovirus vector** comprises a heterologous gene...
...19. The method of claim 1, wherein said **adenovirus vector** further comprises a heterologous gene...

...21. The method of claim 20, wherein said **promoter** is a **CMV promoter**.

4/3,K,AB/3
DIALOG(R)File 340:CLAIMS(R)/US Patent

Dialog Acc No: 3365084 IFI Acc No: 0024873
Document Type: C
GENE THERAPIES FOR ENHANCING CARDIAC FUNCTION; INCREASING CONTRACTILE FUNCTION BY DELIVERING A TRANSGENE ENCODING AN ANGIOGENIC PROTEIN OR PEPTIDE TO THE MYOCARDIUM BY INTRODUCING A REPLICATION-DEFICIENT **ADENOVIRUS VECTOR** INTO THE LUMEN OF A CORONARY ARTERY SUPPLYING BLOOD TO MYOCARDIUM
Inventors: Dillmann Wolfgang H (US); Giordano Frank J (US); Hammond H Kirk (US)
Assignee: California, University of Regents
Assignee Code: 13234
Publication (No,Date), Applic (No,Date):
US 6100242 **20000808** US 97722271 19971229
Publication Kind: A
Calculated Expiration: 20150228
(Cited in 001 later patents) Document Type: CERTIFICATE OF CORRECTION
Certificate of Correction Date: 20010814
Cont.-in-part Pub(No),Applic(No,Date): ABANDONED US 95485472 19950607 US 95396207 19950228; US 5792453
PCT Pub(No,Date),Applic(No,Date): WO 9626742 **19960906** WO 96US2631 19960227
Section 371: 19971229
Section 102(e):19971229
Priority Applic(No,Date): US 97722271 19971229; US 95396207 19950228; US 95485472 19950607

Abstract: The transgene-inserted replication-deficit **adenovirus vector** is effectively used in in vivo gene therapy for peripheral vascular disease and heart disease, including myocardial ischemia, by a single intra-femoral artery or intracoronary injection directly conducted deeply in the lumen of the one or both femoral or coronary arteries (or graft vessels) in an amount sufficient for transfecting cells in a desired region.

...TRANSGENE ENCODING AN ANGIOGENIC PROTEIN OR PEPTIDE TO THE MYOCARDIUM BY INTRODUCING A REPLICATION-DEFICIENT **ADENOVIRUS VECTOR** INTO THE LUMEN OF A CORONARY ARTERY SUPPLYING BLOOD TO MYOCARDIUM
Publication (No,Date), Applic (No,Date):
...**20000808**
...PCT Pub(No,Date),Applic(No,Date): **19960906**

Abstract: The transgene-inserted replication-deficit **adenovirus vector** is effectively used in in vivo gene therapy for peripheral vascular disease and heart disease...

Exemplary Claim: ...angiogenic protein or peptide to the myocardium of the patient by introducing a replication-deficient **adenovirus vector** comprising the transgene into the lumen of a coronary artery supplying blood to the myocardium...
Non-exemplary Claims: ...7. The method of claim 1, wherein about 107 to about 1013 **adenovirus vector** particles are delivered in vivo ...

...8. The method of claim 5, wherein about 109 to about 1012 **adenovirus vector** particles are delivered in vivo...

...9. The method of claim 1, wherein expression of said transgene is driven

by a **CMV promoter** which is contained in the vector... angiogenic protein or peptide to the myocardium of the patient by introducing a replication-deficient **adenovirus vector** comprising the transgene into the lumen of a coronary artery supplying blood to the myocardium...36. The method of claim 30, wherein about 107 to about 1013 **adenovirus vector** particles are delivered in vivo...

...37. The method of claim 34, wherein about 109 to about 1012 **adenovirus vector** particles are delivered in vivo...

...38. The method of claim 30, wherein expression of said transgene is driven by a **CMV promoter** which is contained in the vector... angiogenic protein or peptide to the myocardium of the patient by introducing a replication-deficient **adenovirus vector** comprising the transgene into the lumen of a coronary artery supplying blood to the myocardium...64. The method of claim 58, wherein about 107 to about 1013 **adenovirus vector** particles are delivered in vivo...

...65. The method of claim 61, wherein about 109 to about 1012 **adenovirus vector** particles are delivered in vivo...

...66. The method of claim 58, wherein expression of said transgene is driven by a **CMV promoter** which is contained in the vector... angiogenic protein or peptide to the myocardium of the patient by introducing a replication-deficient **adenovirus vector** comprising the transgene into the lumen of a coronary artery supplying blood to the myocardium...92. The method of claim 86, wherein about 107 to about 1013 **adenovirus vector** particles are delivered in vivo...

...93. The method of claim 90, wherein about 109 to about 1012 **adenovirus vector** particles are delivered in vivo...

...94. The method of claim 86, wherein expression of said transgene is driven by a **CMV promoter** which is contained in the vector...

...95. The method of claim 90, wherein expression of said transgene is driven by a **CMV promoter** which is contained in the vector...

4/3,K,AB/4
DIALOG(R)File 340:CLAIMS(R)/US Patent

Dialog Acc No: 3207761 IFI Acc No: 9931384
Document Type: C
MAMMALIAN TELOMERASE; NUCLEIC ACIDS COMPRISING THE RNA COMPONENT OF A MAMMALIAN TELOMERASE ARE USEFUL AS PHARMACEUTICAL, THERAPEUTIC, AND DIAGNOSTIC REAGENTS.
Inventors: Andrews William H (US); Feng Junli (US); Funk Walter (US); Villeponteau Bryant (US)
Assignee: Geron Corp
Assignee Code: 37860
Publication (No,Date), Applic (No,Date):
US 5958680 **19990928** US 95472802 19950607
Publication Kind: A
Calculated Expiration: 20160928
Cont.-in-part Pub(No),Applic(No,Date): ABANDONED US 94272102 19940707; US 5583016 US 94330123 19941027
Priority Applic(No,Date): US 95472802 19950607; US 94272102 19940707; US 94330123 19941027

Abstract: Nucleic acids comprising the RNA component of a mammalian telomerase are useful as pharmaceutical, therapeutic, and diagnostic reagents.

Publication (No,Date), Applic (No,Date):
...**19990928**
Non-exemplary Claims: ...14. The method of claim 10 wherein the expression **vector** is an **adenovirus**-based **vector**.
...

...metallothionein promoter, constitutive adenovirus major late promoter, dexamethasone-inducible MMTV promoter, SV40 promoter, MRP polIII **promoter**, constitutive MPSV **promoter**, tetracycline-inducible **CMV promoter**, and constitutive **CMV promoter**...
32. The method of claim 29 wherein the expression **vector** is an **adenovirus**-based **vector**.
...

...metallothionein promoter, constitutive adenovirus major late promoter, dexamethasone-inducible MMTV promoter, SV40 promoter, MRP polIII **promoter**, constitutive MPSV **promoter**, tetracycline-inducible **CMV promoter**, and constitutive **CMV promoter**.

4/3,K,AB/5
DIALOG(R)File 340:CLAIMS(R)/US Patent

Dialog Acc No: 3179202 IFI Acc No: 9923813
Document Type: C
RECOMBINANT ADENOVIRAL VECTOR AND METHODS OF USE; PARTIAL OR TOTAL DELETION OF THE ADENOVIRAL PROTEIN IX DNA AND HAVING A GENE ENCODING A FOREIGN PROTEIN OR A FUNCTIONAL FRAGMENT OR MUTANT THEREOF.
Inventors: Gregory Richard J (US); Maneval Daniel C (US); Wills Ken N (US)
Assignee: Canji Inc
Assignee Code: 44322
Publication (No,Date), Applic (No,Date):
US 5932210 **19990803** US 97959638 19971028
Publication Kind: A
Calculated Expiration: 20131025
(Cited in 003 later patents)
Continuation Pub(No),Applic(No,Date): US 94328673 19941025
Cont.-in-part Pub(No),Applic(No,Date): ABANDONED US 93142669 19931025; ABANDONED US 94246006 19940519
Priority Applic(No,Date): US 97959638 19971028; US 94328673 19941025; US 93142669 19931025; US 94246006 19940519

Abstract: This invention provides a recombinant **adenovirus** expression **vector** characterized by the partial or total deletion of the adenoviral protein IX DNA and having a gene encoding a foreign protein or a functional fragment or mutant thereof. Transformed host cells and a method of producing recombinant proteins and gene therapy also are included within the scope of this invention. Thus, for example, the adenoviral vector of this invention can contain a foreign gene for the expression of a protein effective in regulating the cell cycle, such as p53, Rb, or mitosin, or in inducing cell death, such as the conditional suicide gene thymidine kinase. (The latter must be used in conjunction with a thymidine kinase metabolite in order to be effective).

Publication (No,Date), Applic (No,Date):
...**19990803**

Abstract: This invention provides a recombinant **adenovirus** expression **vector** characterized by the partial or total deletion of the adenoviral protein IX DNA and having...

Exemplary Claim: D R A W I N G

1. A composition comprising a recombinant **adenovirus** expression **vector** and a pharmaceutically acceptable carrier, the vector comprising: (a) an insert of exogenous DNA comprising...

Non-exemplary Claims: ...The composition of claim 1, wherein the protein IX polyadenylation site is deleted from the **adenovirus vector**.

...

...1, wherein the gene encoding the foreign protein is expressed under control of a cytomegalovirus (**CMV**) **promoter**.

...

...14. The composition of claim 1, further comprising a host cell transformed with the **adenovirus vector**.

...

...18. The composition of claim 9, wherein the **CMV promoter** is the **CMV** immediate early **promoter**.

4/3,K,AB/6
DIALOG(R)File 340:CLAIMS(R)/US Patent

Dialog Acc No: 3022846 IFI Acc No: 9827622
Document Type: C
GENE TRANSFER-MEDIATED ANGIOGENESIS THERAPY; TRANSFECTING CELLS IN CORONARY ARTERIES BY INJECTION OF A TRANSGENE-INSERTED REPLICATION-DEFICIT ADENOVIRUS; ANTIISCHEMIC AGENTS; CARDIOTONIC AGENTS; VASCULAR DISEASE
Inventors: Dillmann Wolfgang H (US); Giordano Frank J (US); Hammond H Kirk (US)
Assignee: California, University of Regents
Assignee Code: 13234
Publication (No,Date), Applic (No,Date):
US 5792453 **19980811** US 95485472 19950607
Publication Kind: A
Calculated Expiration: 20150811
(Cited in 015 later patents)
Cont.-in-part Pub(No),Applic(No,Date): ABANDONED US 95396207 19950228
Priority Applic(No,Date): US 95485472 19950607; US 95396207 19950228

Abstract: The transgene-inserted replication-deficit **adenovirus vector** is effectively used in in vivo gene therapy for peripheral vascular disease and heart disease, including myocardial ischemia, by a single intra-femoral artery or intracoronary injection directly conducted deeply in the lumen of the one or both femoral or coronary arteries (or graft vessels) in an amount sufficient for transfecting cells in a desired region.

Publication (No,Date), Applic (No,Date):
...**19980811**

Abstract: The transgene-inserted replication-deficit **adenovirus vector** is effectively used in in vivo gene therapy for peripheral vascular disease and heart disease...

Exemplary Claim: ...A method for stimulating coronary collateral vessel development in a patient, comprising delivering a replicationdeficient **adenovirus vector** to the myocardium of the patient by intracoronary injection directly into one or both coronary...
Non-exemplary Claims: ...3. The method of claim 1, wherein about 107 to about 1013 **adenovirus vector** particles are delivered in the injection...

...4. The method of claim 1, wherein about 109 to about 1012 **adenovirus vector** particles are delivered in the injection ...

...5. The method of claim 1, wherein about 1011 **adenovirus vector** particles are delivered in the injection...

...6. The method according to claim 1, wherein said transgene is driven by a **CMV promoter** which is contained in the vector...vessel development in a patient having peripheral-deficient vascular disease, comprising delivering a replication-deficient **adenovirus vector** to the peripheral vascular system of the patient by intra-femoral artery injection directly into...

...21. The method of claim 19, wherein about 109 to about 1013 **adenovirus vector** particles are delivered in the injection ...
...22. The method of claim 19, wherein about 109 to about 1012 **adenovirus vector** particles are delivered in the injection ...

...23. The method of claim 19, wherein about 1011 **adenovirus vector** particles are delivered in the injection...

...25. A method for treating heart myocardial ischemia, comprising delivering a replication-deficient **adenovirus vector** to the myocardium of a patient by intracoronary injection, said vector comprising a transgene coding...

4/3,K,AB/7
DIALOG(R)File 340:CLAIMS(R)/US Patent

Dialog Acc No: 2695930 IFI Acc No: 9605155
Document Type: C
BROAD-SPECTRUM TUMOR SUPPRESSOR GENES, GENE PRODUCTS AND METHODS FOR TUMOR SUPPRESSOR GENE THERAPY; ANTI-ONCOGENES
Inventors: Benedict William F (US); Hu Shi-Xue (US); Xu Hong-Ji (US)
Assignee: Unassigned Or Assigned To Individual
Assignee Code: 68000 Document Type: REASSIGNED
Publication (No,Date), Applic (No,Date):
US 5496731 **19960305** US 9338760 19930325
Publication Kind: A
Calculated Expiration: 20130325
(Cited in 014 later patents)
Priority Applic(No,Date): US 9338760 19930325

Abstract: The present invention relates to a broad-spectrum tumor suppressor gene and the protein expressed by that gene in appropriate host cells. The protein is a second in-frame AUG codon-initiated retinoblasoma protein of about 94 kD relative molecular mass. The present invention also relates to methods of treating a mammal having a disease or disorder characterized by abnormal cellular proliferation, such as a tumor or cancer and methods of treating abnormally proliferating cells, such as tumor or cancer cells. Treatment is accomplished by inserting a host cell compatible

p94RB expression vector or an effective amount of p94RB protein into a cell or cells in need of treatment.

Publication (No,Date), Applic (No,Date):
...**19960305**
Non-exemplary Claims: ...is under the control of a promoter selected from the group consisting of a retroviral **promoter**, a **CMV promoter** and a Beta -actin **promoter**.
...

...9. The expression vector according to claim 3 wherein said expression **vector** is an **adenovirus** and said p94RB encoding gene is under the control of a promoter selected from the group consisting of an adenoviral **promoter**, a **CMV promoter** and a Beta -actin **promoter**.
?

§1.136(a), and any fees required therefore are hereby authorized to be charged to our Deposit Account 20-0823.

IN THE CLAIMS:

Please cancel claims 1-5, 10, and 16-30.

The following claims have been amended as reflected in a separate marked-up version of the claims attached hereto:

6. (Amended) An isolated and purified polynucleotide comprising a nucleotide sequence encoding an isolated and purified polypeptide wherein the polypeptide (a) is a variant type 2 methionine aminopeptidase ("MetAP2"), (b) has dominant negative MetAP2 activity and (c) contains a translation domain.

7. (Amended) The isolated and purified polynucleotide of claim 6 wherein the isolated and purified polynucleotide encodes a peptide that comprises SEQ ID NO:6 or a fragment thereof.

8. (Amended) The isolated and purified polynucleotide of claim 7 wherein the isolated and purified polynucleotide encodes a peptide that consists essentially of SEQ ID NO:6 or a fragment thereof.

9. (Amended) The isolated and purified polynucleotide of claim 8 wherein the isolated and purified polynucleotide comprises SEQ ID NO:9.

11. (Amended) A vector containing an isolated and purified polynucleotide of claim 6.

We claim:

1. An isolated and purified polypeptide wherein the polypeptide (a) is a variant type 2 methionine aminopeptidase ("MetAP2"), (b) has dominant negative MetAP2 activity and (c) contains a translation domain.

2. The isolated and purified polypeptide of claim 1 comprising a sequence which is at least 46% identical to SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8 or SEQ ID NO:16, or a fragment thereof.

3. The isolated and purified polypeptide of claim 2 comprising SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8 or SEQ ID NO:16, or a fragment thereof.

4. The isolated and purified polypeptide of claim 3 which consists essentially of SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8 or SEQ ID NO:16, or a fragment thereof.

5. The isolated and purified polypeptide of claim 4 which consists essentially of SEQ ID NO:12, wherein the histidine at position number 231 is replaced with an alanine.

6. An isolated and purified polynucleotide comprising a nucleotide sequence encoding the polypeptide of claim 1.

7. The isolated and purified polynucleotide of claim 6 wherein the isolated and purified polynucleotide encodes a peptide that comprises SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8 or SEQ ID NO:16, or a fragment thereof.

8. The isolated and purified polynucleotide of claim 7 wherein the isolated and purified polynucleotide encodes a peptide that consists essentially of SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8 or SEQ ID NO:16, or a fragment thereof.

9. The isolated and purified polynucleotide of claim 8 wherein the isolated and purified polynucleotide comprises a sequence selected from the list consisting of SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11 and SEQ ID NO:18.

10. The isolated and purified polynucleotide of claim 9 wherein the purified polynucleotide comprises SEQ ID NO:9.

11. A vector containing an isolated and purified polynucleotide which encodes the polypeptide of claim 1.

12. The vector of claim 11 wherein the polypeptide is SEQ ID NO:6.

13. The vector of claim 12 wherein the polynucleotide consists of SEQ ID NO:9.

14. The vector of claim 13 wherein the polynucleotide is operably linked to a promoter which is selected from the list consisting of GAL1, CMV, GPD, an endothelial cell-specific promoter and an immune cell-specific promoter.

15. The vector of claim 14 wherein the vector is an adenovirus and the promoter is CMV.

16. A method of treating a cell comprising contacting the cell with a composition comprising an isolated and purified polypeptide, wherein the polypeptide is a variant MetAP2 that has dominant negative MetAP2 activity and contains a translation domain.

17. The method of claim 16 wherein the cell is in a subject.

18. The method of claim 17 wherein the subject suffers from a disease mediated by a fungal infection/cell proliferation, angiogenesis/decreased function of p53 /or immune system activity.

19. The method of claim 18 wherein the subject is a human suffering from a disease mediated by angiogenesis.

20. A method of treating a cell comprising contacting the cell with a composition comprising an isolated and purified polynucleotide, wherein the polynucleotide encodes a variant MetAP2 that has dominant negative methionine MetAP2 activity and contains a translation domain.

22. The method of claim 21 wherein the cell is in a subject.

23. The method of claim 22 wherein the subject is a human patient suffering from a disease mediated by fungal infection, cell proliferation, angiogenesis, decreased function of p53 or immune system activity.

24. The method of claim 23 wherein the disease is a disease mediated by angiogenesis.

25. The method of claim 24 wherein the polynucleotide is part of an adenovirus vector and operably linked to a CMV promoter.

26. A method of identifying an agent that modulates the activity of MetAP2 comprising contacting a cell with the agent, wherein

(a) the cell contains a functional gene that encodes a MetAP2 and does not contain an operable naturally occurring chromosomal copy of a gene encoding a MetAP1, and

(b) the modulation activity of the agent is determined by measuring either the relative growth rate of the cell or the fluorescence emission of the cell.

27. The method of claim 26 wherein

(a) the cell is a yeast cell which comprises a gene encoding MetAP1 operably linked to a regulatable promoter, and

(b) the modulation activity of the agent is determined by comparing the growth rate of the yeast cell in the absence of MetAP1 expression to the growth rate of the yeast cell in the presence of MetAP1 expression.

28. The method of claim 26 wherein

(a) the cell is a mammalian cell which comprises a polynucleotide that further comprises a gene encoding a MetAP1 and a gene encoding a fluorescent protein, and

(b) the modulation activity of the agent is determined by measuring the fluorescence emission of the cell.

29. The method of claim 25 wherein the agent is a polynucleotide.

30. A method of identifying effectors of MetAP2 activity comprising contacting a yeast cell with a polynucleotide and determining that the polynucleotide encodes an effector of MetAP2 activity, wherein

(a) the yeast cell comprises a functional gene that encodes a MetAP2 and a polynucleotide that encodes a dominant negative MetAP2,

(b) the yeast cell does not contain an operable naturally occurring chromosomal copy of a gene encoding a MetAP1, and

(c) the determining step comprises comparing the growth rate of yeast cells that contains a polynucleotide that encodes an effector of MetAP2 activity with a yeast cell that does not contain a polynucleotide that encodes an effector of MetAP2 activity, wherein the growth rate of a yeast cell that contains a polynucleotide that encodes an effector of MetAP2 activity is greater than the growth rate of a yeast cell that does not contain a polynucleotide that encodes an effector of MetAP2 activity.

31. The method of claim 30 wherein the polynucleotide is a human polynucleotide.

DETAILED ACTION

It is noted that claims 22-31 were misnumbered and have been renumbered as claims 21-30, according to rule 126.

Election/Restrictions

Restriction to one of the following inventions is required under 35 U.S.C. 121:

Groups 1-5. Claims 1-5, drawn to a variant polypeptide of type 2 methionine aminopeptidase (MetAP2) that has dominant negative MetAP2 activity, comprising SEQ ID NO:6, 7, 8, 16 or SEQ ID NO:12 wherein the histidine at position 231 is replaced with alanine, classified in class 530, subclass 350. Each sequence constitutes a single invention and not a species.

Groups 6-9. Claims 6-15, drawn to a polynucleotide of SEQ ID NO: 9, 10, 11 or 18, encoding SEQ ID NO:6, 7, 8, 16, and a vector containing said polynucleotide, classified in class 536, subclass 23.1. Each sequence constitutes a single invention and not a species.

Groups 10-14. Claims 16-19, drawn to a method for treating fungal infection, comprising administering a variant polypeptide of type 2 methionine aminopeptidase (MetAP2) that has dominant negative MetAP2 activity, comprising SEQ ID NO:6, 7, 8, 16 or SEQ ID NO:12 wherein the histidine at position 231 is replaced with alanine, classified in class 514, subclass 2. Each method of treatment using each sequence constitutes a single invention and not a species.

Groups 15-19. Claims 16-19, drawn to a method for treating cell proliferation, comprising administering a variant polypeptide of type 2 methionine aminopeptidase

(MetAP2) that has dominant negative MetAP2 activity, comprising SEQ ID NO:6, 7, 8, 16 or SEQ ID NO:12 wherein the histidine at position 231 is replaced with alanine, classified in class 514, subclass 2. Each method of treatment using each sequence constitutes a single invention and not a species.

Groups 20-24. Claims 16-19, drawn to a method for treating angiogenesis, comprising administering a variant polypeptide of type 2 methionine aminopeptidase (MetAP2) that has dominant negative MetAP2 activity, comprising SEQ ID NO:6, 7, 8, 16 or SEQ ID NO:12 wherein the histidine at position 231 is replaced with alanine, classified in class 514, subclass 2. Each method of treatment using each sequence constitutes a single invention and not a species.

Groups 25-29. Claims 16-19, drawn to a method for treating a disease mediated by decreased function of p53, comprising administering a variant polypeptide of type 2 methionine aminopeptidase (MetAP2) that has dominant negative MetAP2 activity, comprising SEQ ID NO:6, 7, 8, 16 or SEQ ID NO:12 wherein the histidine at position 231 is replaced with alanine, classified in class 514, subclass 2. Each method of treatment using each sequence constitutes a single invention and not a species.

Group 30-34. Claims 16-19, drawn to a method for treating a disease mediated by immune system activity, comprising administering a variant polypeptide of type 2 methionine aminopeptidase (MetAP2) that has dominant negative MetAP2 activity, comprising SEQ ID NO:6, 7, 8, 16 or SEQ ID NO:12 wherein the histidine at position 231 is replaced with alanine, classified in class 514, subclass 2. Each method of treatment using each sequence constitutes a single invention and not a species.

Groups 35-39. Claims 20-24, drawn to a method for treating fungal infection, comprising administering a polynucleotide encoding a variant polypeptide of type 2 methionine aminopeptidase (MetAP2) that has dominant negative MetAP2 activity, comprising SEQ ID NO:6, 7, 8, 16 or SEQ ID NO:12 wherein the histidine at position 231 is replaced with alanine, classified in class 514, subclass 44. Each method of treatment using each sequence constitutes a single invention and not a species.

Groups 40-44. Claims 20-24, drawn to a method for treating cell proliferation, comprising administering a polynucleotide encoding a variant polypeptide of type 2 methionine aminopeptidase (MetAP2) that has dominant negative MetAP2 activity, comprising SEQ ID NO:6, 7, 8, 16 or SEQ ID NO:12 wherein the histidine at position 231 is replaced with alanine, classified in class 514, subclass 44. Each method of treatment using each sequence constitutes a single invention and not a species.

Groups 45-49. Claims 20-24, drawn to a method for treating angiogenesis, comprising administering a polynucleotide encoding a variant polypeptide of type 2 methionine aminopeptidase (MetAP2) that has dominant negative MetAP2 activity, comprising SEQ ID NO:6, 7, 8, 16 or SEQ ID NO:12 wherein the histidine at position 231 is replaced with alanine, classified in class 514, subclass 44. Each method of treatment using each sequence constitutes a single invention and not a species.

Groups 50-54. Claims 20-24, drawn to a method for treating a disease mediated by decreased function of p53, comprising administering a polynucleotide encoding a variant polypeptide of type 2 methionine aminopeptidase (MetAP2) that has dominant negative MetAP2 activity, comprising SEQ ID NO:6, 7, 8, 16 or SEQ ID NO:12 wherein

the histidine at position 231 is replaced with alanine, classified in class 514, subclass 44. Each method of treatment using each sequence constitutes a single invention and not a species.

Groups 55-59. Claims 20-24, drawn to a method for treating a disease mediated by immune system activity, comprising administering a pollynucleotide encoding a variant polypeptide of type 2 methionine aminopeptidase (MetAP2) that has dominant negative MetAP2 activity, comprising SEQ ID NO:6, 7, 8, 16 or SEQ ID NO:12 wherein the histidine at position 231 is replaced with alanine, classified in class 514, subclass 44. Each method of treatment using each sequence constitutes a single invention and not a species.

Group 60. Claims 25, 28, drawn to a method for identifying an agent that modulates the activity of MetAP2, using a cell that contains a functional gene that encodes a MetAP2, and wherein said cell does not contain an operable naturally occurring chromosomal copy of a gene encoding a MetAP1, classified in class 435, subclass 4.

Group 61. Claim 26, drawn to a method for identifying an agent that modulates the activity of MetAP2, using a yeast cell which comprises a gene encoding MetAP1 operably linked to a regulatory promoter, classified in class 435, subclass 4.

Groups 62. Claim 27, drawn to a method for identifying an agent that modulates the activity of MetAP2, using a mammalian cell which comprises a gene encoding MetAP1 and a gene encoding a fluorescent protein, classified in class 435, subclass 4.

Groups 63-67. Claims 29-30, drawn to a method for identifying effectors of MetAP2 activity, using a yeast cell that comprises a functional gene that encodes a MetAP2 and a polynucleotide that encodes a dominant negative MetAP2, of SEQ ID NO:6, 7, 8, 16 or SEQ ID NO:12 wherein the histidine at position 231 is replaced with alanine, and wherein said yeast cell does not contain an operable naturally occurring chromosomal copy of a gene encoding a MetAP1, classified in class 435, subclass 4.. Each method of treatment using each sequence that encodes a single dominant negative MetAP2 constitutes a single invention and not a species.

In addition upon election of any of groups 60, 62, further election of the following species is required:

Measuring cell growth or fluorescence emission.

The inventions are distinct, each from each other because of the following reasons:

Inventions (1-9) and (10-67) are related as product and process of use. The inventions can be shown to be distinct if either or both of the following can be shown: (1) the process for using the product as claimed can be practiced with another materially different product or (2) the product as claimed can be used in a materially different process of using that product (M.P.E.P. 806.05 (h). In this instant case, a polypeptide could be used for several purposes, e.g. for biochemical assay, for making antibodies, and for making an affinity column to purify its antibodies; a DNA sequence could be used for the detection of similar DNA or RNA sequences, for making an expression vector, and for producing its encoded protein.

The products of groups (1-9) are patentably distinct, because they are drawn to entirely different biochemicals , having different structures.

The methods of groups (10-67) are distinct from each other because they differ at least in objectives, method steps, reagents and/or dosages, and/or schedules used, response variables and criteria for success.

The species measuring cell growth or fluorescence emission are distinct because they are different methods having different method steps, reagents and/or dosages, and/or schedules used, response variables and criteria for success.

Because these inventions are distinct for the reason given above and have acquired a separate status in the art, and because the searches for the groups are not co-extensive, restriction for examination purposes as indicated is proper.

Applicants are required under 35 USC 121 to elect a single disclosed group for prosecution on the merits to which the claims shall be restricted. Applicant is further advised that if Applicant elects a group having species requirement, a response to this requirement must include an identification of the species that is elected consonant with this requirement, and a listing of all claims readable thereon, including any claims subsequently added.

Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which are written in dependent form or otherwise include all the limitations of an allowed generic claim as provided by 37 CFR 1.141. If claims are added after the election, applicant must indicate which are readable upon the elected species. MPEP 809.02(a).

Should applicant traverse on the ground that the species are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the species to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the inventions unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 USC 103 of the other invention.

Applicants are reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 C.F.R. 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendement of inventorship must be accompanied by a diligently-filed petition under 37 C.F.R. 1.48(b) and by the fee required under 37 C.F.R. 1.17(h).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to MINH-TAM DAVIS whose telephone number is 703-305-2008. The examiner can normally be reached on 9:30AM-4:00PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, ANTHONY CAPUTA can be reached on 703-308-3995. The fax phone numbers for the organization where this application or proceeding is assigned are 703-872-9306 for regular communications and 703-872-9307 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703-308-0916.

MINH TAM DAVIS

November 02/ 2002

DETAILED ACTION

Applicant's election with traverse of group 6, claims 6-15, SEQ ID NO:9, in Paper No. 10 is acknowledged.

In a telephonic conversation with Kimberly Lu on 01/30/03, Applicant elected the species 1) CMV promoter and 2) inhibition of cell proliferation of the generic dominant negative MetAP2 activity, which includes inhibition of the cleavage of the N-terminal methionine residue from nascent peptides, promotion of cell proliferation, angiogenesis, immune system function, and the inhibition of p53 activity, but not the regulation of protein synthesis, as disclosed in the specification on p.10, second paragraph.

It is noted that SEQ ID NO:9 is a polynucleotide encoding a variant of human MetAP2, wherein His 231 is replaced with Ala (specification, page 5, lines 25-26). Thus the amino acid sequence encoded by SEQ ID NO:9 is SEQ ID NO:6 wherein the designation Xaa at position 231 is Ala, and other amino acids designated as Xaa are the same as the wild type; which is the same as SEQ ID NO:12 (wild type human MetAP2) wherein His231 of SEQ ID NO:12 is replaced with Ala231.

Applicant cancels claim 10 and non elected claims 1-5, 16-30.

Accordingly, claims 6- 9, 11-15, SEQ ID NO:9, species inhibition of cell proliferation, and CMV promoter, are examined in the instant application. Claims 7-8, 12, drawn to a polynucleotide encoding SEQ ID NO:6 is examined only to the extent of a polynucleotide encoding SEQ ID NO:6, wherein the designation Xaa at position 231 of SEQ ID NO:6 is Ala, and wherein other amino acids designated as Xaa in SEQ ID NO:6 are the same as the wild type amino acids of the human MetAP2. In other word, the

examined SEQ ID NO:6 is the same as SEQ ID NO:12 (wild type human MetAP2), wherein His231 of SEQ ID NO:12 is replaced with Ala231.

OBJECTION

1. Claims 6-8, 12 are objected to because part of claims 6-8, 12 encompasses polynucleotides encoding variants of MetAP2 that are not elected, e.g. SEQ ID NO: 10, 11, 18, and polynucleotides encoding SEQ II NO:6, wherein amino acids designated as Xaa in positions other than position 231 are any amino acids other than the wild type amino acids.

2. Claims 7 and 8 are objected to because they are drawn to the same composition. Claim 8 is drawn to a peptide that "consists essentially" of SEQ ID NO:6. The language "consists essentially" of claim 8 is interpreted to mean the same as "comprises" of claim 7.

Applicant is advised that should claim 7 be found allowable, claim 8 will be rejected under 35 U.S.C. 101 as being a substantial duplicate thereof, When two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to reject the other as being a substantial duplicate of the allowed claim. See MPEP 706.03(k).

REJECTION UNDER 35 USC 112, FIRST PARAGRAPH, WRITTEN DESCRIPTION

The instant specification does not contain a written description of the invention in such full, clear, concise, and exact terms or in sufficient detail that one skilled in the art can reasonably conclude that applicant had possession of the claimed invention at the time of filing.

The claims 7 and 8 are drawn to a polynucleotide encoding a peptide "comprising" a fragment of SEQ ID NO:6.

It is noted that a polynucleotide encoding a peptide comprising a fragment of SEQ ID NO:6 encompasses a polynucleotide of any structure and any length, provided it encodes peptide comprising a fragment of SEQ ID NO:6, wherein said fragment could be as little as two amino acids and does not necessarily have cell proliferation inhibiting property.

The specification discloses the variant human polypeptide MetAP2 of SEQ ID NO:6 (p.16, first paragraph).

The claims, as written, encompass polynucleotides which vary substantially in length and also in nucleotide composition.

The instant disclosure of a single species of nucleic acid does not adequately describe the scope of the claimed genus, which encompasses a substantial variety of subgenera including full-length genes. A description of a genus of cDNAs may be acheived by means of a recitation of a representative number of cDNAs, defined by nucleotide sequence, falling within the scope of the genus or of a recitation of structural freatures common to members of the genus, which features constitute a substantial portion of the genus. *Regents of the University of California v. Eli Lilly & Co.*, 119 F3d

1559, 1569, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997). The instant specification fails to provide sufficient descriptive information, such as definitive strutural or functional features of the claimed genus of polynucleotides. There is no description of the conserved regions which are critical to the structure and function of the genus claimed. There is no description, however, of the sites at which variability may be tolerated and there is no information regarding the relation of structure to function. Structural freatures that could distinguish the compounds in the genus from others excluded are missing from the disclosure. Furthermore, the prior art does not provide compensatory structural or correlative teachings sufficient to enable one of skill to isolate and identify the polynucleotides encompassed and no identifying characteristic or property of the instant polynucleotides is provided such that one of skill would be able to predictably identify the encompassed molecules as being identical to those instantly claimed.

Since the disclosure fails to describe the common attributes or characteristics that identify members of the genus, and because the genus is highly variant, the disclosure of specific nucleotide sequences and the ability to screen, is insufficient to describe the genus. One of skill in the art would reasonably conclude that the disclosure fails to provide a representative number of species to describe and enable the genus as broadly claimed. Thus, only the isolated polynucleotide of SEQ ID NO: 9, encoding SEQ ID NO: 6, but not the full breadth of the claims meet the written description provisions of 35 USC 112, first paragraph.

REJECTION UNDER 35 USC 112, FIRST PARAGRAPH, SCOPE

Claims 6-9, 11-15 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for SEQ ID NO:9, encoding a protein which inhibits endothelial cell proliferaton *in vitro*, does not reasonably provide enablement for a polynucleotide encoding a variant type 2 methionine amino peptidase which inhibits cell proliferation *in vivo*. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Claims 6-9, 11-15 are drawn to a polynucleotide encoding a variant type 2 methionine amino peptidase, which inhibits cell proliferation and contains a translation domain, wherein said polynucleotide comprises SEQ ID NO:9.

Claims 6-9, 11-15 encompass a polynucleotide encoding a variant type 2 methionine amino peptidase, which inhibits cell proliferation "in vivo", e.g. cancer cell proliferation.

The specification discloses that a vector comprising SEQ ID NO:9 (AdMAP2 (H231A), when transfected into umbilical vascular endothelial cells *in vitro* inhibits cell proliferation (Example 3, page 34).

One cannot extrapolate the teaching of the specification to the claimed invention because there is no guidance on or exemplification of any correlation between inhibition of proliferation of cells transfected with SEQ ID NO:9 and *in vivo* inhibition of cell proliferation. The *in vitro* transfection data presented is clearly not drawn to subjects with tumor cells, wherein in transfected cells usually the protein is artificially overexpressed, which is not the same conditions as in vivo conditions. Further,

characteristics of cultured cell lines generally differ significantly from the characteristics of a primary tumor. Drexler et al (Leukemia and Lymphoma, 1993, 9:1-25) specifically teach, in the study of Hodgkin and Reed-Sternberg cancer cells in culture, that the acquisition or loss of certain properties during adaptation to culture systems cannot be excluded and that only a few cell lines containing cells that resemble the *in-vivo* cancer cells have been established and even for the *bona fide* cancer cell lines it is difficult to prove that the immortalized cells originated from a specific cancer cell (see attached abstract). Further, Embleton et al (Immunol Ser, 1984, 23:181-207) specifically teaches that in procedures for the diagnosis of osteogenic sarcoma, caution must be used when interpreting results obtained with monoclonal antibodies that had been raised to cultured cell lines and specifically teach that cultured tumor cells may not be antigenically typical of the tumor cell population from which they were derived and it is well established that new artifactural antigens can occur as a result of culture (see attached abstract). Hsu (in Tissue Culture Methods and Applications, Kruse and Patterson, Eds, 1973, Academic Press, NY, see abstract, p.764) specifically teaches that it is well known that cell cultures *in vitro* frequently change their chromosomal constitutions (see abstract). The evidence presented clearly demonstrates that in cell culture systems, in general, and in cancer derived cell lines in particular, that artifactural chromosome constitutions and antigen expression are expected and must be taken into account when interpreting data received from cell line assays. Further, Freshney (Culture of Animal Cells, A Manual of Basic Technique, Alan R. Liss, Inc., 1983, New York, p4) teach that it is recognized in the art that there are many differences between cultured cells and their

counterparts *in vivo*. These differences stem from the dissociation of cells from a three-dimensional geometry and their propagation on a two-dimensional substrate. Specific cell interactions characteristic of histology of the tissue are lost. The culture environment lacks the input of the nervous and endocrine systems involved in homeostatic regulation *in vivo*. Without this control, cellular metabolism may be more constant *in vitro* but may not be truly representative of the tissue from which the cells were derived. This has often led to tissue culture being regarded in a rather skeptical light (p. 4, see Major Differences *In Vitro*). Further, Dermer (Bio/Technology, 1994, 12:320) teaches that, petri dish cancer is a poor representation of malignancy, with characteristics profoundly different from the human disease Further, Dermer teaches that when a normal or malignant body cell adapts to immortal life in culture, it takes an evolutionary -type step that enables the new line to thrive in its artificial environment. This step transforms a cell from one that is stable and differentiated to one that is not, yet normal or malignant cells *in vivo* are not like that. The reference states that evidence of the contradictions between life on the bottom of a lab dish and in the body has been in the scientific literature for more than 30 years. Clearly it is well known in the art that cells in culture exhibit characteristics different from those *in vivo* and cannot duplicate the complex conditions of the *in vivo* environment involved in host-tumor and cell-cell interations. Thus, based on the transfected cell data presented in the specification, it could not be predicted that, in the *in vivo* environment, the variant type 2 methionine amino peptidase encoded by SEQ ID NO:9 would inhibit cell proliferation, such as cell proliferation of cancer cells.

Further, one cannot extrapolate the teaching of the specification to the claims because it is well known that the art of anticancer drug discovery for cancer therapy is highly unpredictable, for example, Gura (Science, 1997, 278:1041-1042) teaches that researchers face the problem of sifting through potential anticancer agents to find ones promising enough to make human clinical trials worthwhile and teach that since formal screening began in 1955, many thousands of drugs have shown activity in either cell or animal models but that only 39 have actually been shown to be useful for chemotherapy (p. 1041, see first and second para). Because of the known unpredictability of the art, in the absence of experimental evidence, no one skilled in the art would accept the assertion that in the *in vivo* environment, the variant type 2 methionine amino peptidase encoded by SEQ ID NO:9 would inhibit cell proliferation, such as cell proliferation of cancer cells. Further, the refractory nature of cancer to drugs is well known in the art. Jain (Sci. Am., 1994, 271:58-65) teaches that tumors resist penetration by drugs (p.58, col 1) and that scientists need to put expanded effort into uncovering the reasons why therapeutic agents that show encouraging promise in the laboratory often turn out to be ineffective in the treatment of common solid tumors (p. 65, col 3). Curti (Crit. Rev. in Oncology/Hematology, 1993, 14:29-39) teaches that solid tumors resist destruction by chemotherapy agents and that although strategies to overcome defense mechanisms of neoplastic cells have been developed and tested in a number of patients, success has been limited and further teaches that it is certainly possible that cancer cells possess many as yet undefined additional molecular mechanisms to defeat chemotherapy treatment strategies and if this is true, designing effective chemotherapeutic regimens

for solid tumors may prove a daunting task (para bridging pages 29-30) and concludes that knowledge about the physical barriers to drug delivery in tumors is a work in progress (p. 36, col 2). It is clear that based on the state of the art, in the absence of experimental evidence, no one skilled in the art would accept the assertion that in the *in vivo* environment, the variant type 2 methionine amino peptidase encoded by SEQ ID NO:9 would inhibit cell proliferation, such as cell proliferation of cancer cells. In addition, Hartwell et al (Science, 1997, 278:1064-1068) teach that an effective chemotherapeutic must selectively kill tumor cells, that most anticancer drugs have been discovered by serendipity and that the molecular alterations that provide selective tumor cell killing are unknown and that even understanding the detailed molecular mechanism by which a drug acts often provides little insight into why the treated tumor cell dies (para bridging pages 1064-1065) and Jain (cited supra) specifically teaches that systemic treatment typically consists of chemotherapeutic drugs that are toxic to dividing cells (p. 58, col 2, para 2).

For the above reasons, it appears that undue experimentation would be required to practice the claimed inventions with a reasonable expectation of success.

REJECTION UNDER 35 USC 102

Claims 6-9 are rejected under 35 USC 102 as being anticipated by Griffith, EC et al, 1998, Proc Natl Acad Sci, USA, 95: 15183-15188, as evidenced by Arfin SM et al, 1995, PNAS USA, 92(17): 7714-7718.

Claims 6-9 are drawn to a polynucleotide encoding a polypeptide wherein said a polypeptide (a) is a variant of type 2 methionine aminopeptidase (MetAP2), (b) inhibits cell proliferation, and contains a translation domain. Said polypeptide comprises SEQ ID NO:6 or a fragment thereof. Said polynucleotide comprises SEQ ID NO:9.

It is noted that the only part of SEQ ID NO:6 that is examined is wild type MetAP2, wherein amino acid Xaa at position 231 is Ala231, and wherein any other Xaa at any other amino acid positions are the same as the wild type amino acids. In other words, the part of SEQ ID NO:6 that is examined is the same as SEQ ID NO:12 (wild type MetAP2), wherein His231 is replaced with Ala231 (p.5, second paragraph).

It is further noted that wild type human polynucleotide MetAP2 and the encoded polypeptide MetAP2 are well known in the art (Arfin SM et al, 1995, PNAS USA, 92(17): 7714-7718 and MPSRCH search reports 2003, us-09-943-123-9.rge, pages 2-3, and us-09-943-123-6.rsp, pages 1-2).

The specification discloses SEQ ID NO:9 is a human polynucleotide MetAP2 encoding the human polypeptide MetAP2, wherein His231 is replaced with Ala231 (p. 20, first paragraph). The specification further discloses that MetAP2 consists of two domains: 1) a conserved C-terminal catalytic domain and an N-terminal polylysine domain predicted to mediate ribosome oreIF2 association, named the translation domain (p.12, lines 31-33).

Griffith, EC et al teach construction of a polynucleotide variant of human MetAP2, by mutagenesis at His231 of MetAP2, and that mutation of His231 to H231A results in its complete loss of catalytic activity of MetAP2 (p. 15184, second paragraph, paragraph

under Construction of human MetAP2 mutants, p. 1586, second column, last pargraph, bridging page 1587 and figure 5 on page 1586).

The reference does not specifically teach that the variant of MetAP2 inhibits cell proliferation and contains a translation domain. However, the claimed MetAP2 variant appears to be the same as the prior art MetAP2 variant. The office does not have the facilities and resources to provide the factual evidence needed in order to establish that the product of the prior art does not possess the same material, structural and functional characteristics of the claimed product. In the absence of evidence to the contrary, the burden is on the applicant to prove that the claimed product is different from those taught by the prior art. See In re Best 562F.2d 1252, 195 USPQ 430 (CCPA 1977) and Ex parte Gray 10 USPQ 2d 1922 (PTO Bd. Pat. App. & Int. 1989).

REJECTION UNDER 35 USC 103

Claims 11-15 are rejected under 35 USC 103 as being obvious over Griffith, EC et al, 1998, Proc Natl Acad Sci, USA, 95: 15183-15188, in view of US 6,110744.

Claims 11-15 are drawn to a vector containing a polynucleotide encoding a polypeptide wherein said polypeptide (a) is a variant of type 2 methionine aminopeptidase (MetAP2), (b) inhibits cell proliferation, and contains a translation domain. Said polypeptide is SEQ ID NO:6 and said polynucleotide is SEQ ID NO:9, which is operably linked to a promoter which is CMV. The vector is adenovirus vector.

The teaching of Griffith, EC et al has been set forth above.

Griffith, EC et al do not teach a vector containing a polynucleotide encoding a polypeptide wherein said polypeptide (a) is a variant of type 2 methionine aminopeptidase (MetAP2), (b) inhibits cell proliferation, and contains a translation domain. Griffith, EC et al do not teach that said polypeptide is SEQ ID NO:6 and said polynucleotide is SEQ ID NO:9, which is operably linked to a promoter which is CMV. Griffith, EC et al do not teach that the vector is adenovirus vector.

US 6,110744 teaches adenovirus vector comprising a heterologuous gene and a promoter which is CMV.

It would have been *prima facia* obvious to a person of ordinary skill in the art at the time the invention was made to clone the polynucleotide sequence taught by Griffth et al in an adenovirus vector having CMV as a promoter, as taught by US 6,110744, because cloning a sequence into a vector is common in the art, and because adenovirus vector comprising a heterologuous gene and a promoter which is CMV is well known in the art.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to MINH-TAM DAVIS whose telephone number is 703-305-2008. The examiner can normally be reached on 9:30AM-4:00PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, ANTHONY CAPUTA can be reached on 703-308-3995. The fax phone numbers for the organization where this application or proceeding is assigned are 703-

872-9306 for regular communications and 703-872-9307 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703-308-0916.

MINH TAM DAVIS

August 4, 2003